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Mineral
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VOLUME I

PRINCIPLES, PROCESSES, AND SYSTEMS

PART A

Volume I

PRINCIPLES, PROCESSES, AND SYSTEMS

In Two Parts

PART A. Chapters 1–10
PART B. Chapters 11–16

Volume II

THE ELEMENTS

In Two Parts

Mineral Metabolism

AN ADVANCED TREATISE

Edited by

C. L. COMAR
Cornell University
Ithaca, New York

FELIX BRONNER
Hospital for Special Surgery
Cornell Medical Center
New York, New York

VOLUME I

PRINCIPLES, PROCESSES, AND SYSTEMS

PART A

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Contributors to Volume I, Part A

EUGENE Y. BERGER, *New York University Research Service, Goldwater Memorial Hospital, New York, New York.*

JAY BOYD BEST, *Department of Neurophysiology, Walter Reed Army Institute of Research, Washington, D.C.*

STANFORD GOLDMAN, *Department of Electrical Engineering, Syracuse University, Syracuse, New York.*

JOHN Z. HEARON, *Office of Mathematical Research, National Institutes of Health, Bethesda, Maryland.*

E. PHILIP HORWITZ, *Dow Chemical Company, Midland, Michigan.*

VALBORG KOEFOED-JOHNSEN, *Institute of Biological Chemistry, University of Copenhagen, Denmark.*

FRANKLIN C. MCLEAN, *Department of Physiology, University of Chicago, Illinois.*

THERALD MOELLER, *Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois.*

GILBERT H. MUDGE, *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland.*

J. R. ROBINSON, *Department of Physiology, University of Otago Medical School, Dunedin, New Zealand.*

IRVING L. SCHWARTZ, *Medical Research Center, Brookhaven National Laboratory, Upton, New York.*

A. K. SOLOMON, *Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts.*

HANS F. USSING, *Institute of Biological Chemistry, University of Copenhagen, Denmark.*

MACKENZIE WALSER, *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland.*

Contributors to Volume I, Part B

GÖRAN C. H. BAUER, *Malmö Allmänna Sjukhus, Malmö, Sweden.*

ARVID CARLSSON, *Institute of Pharmacology, University of Lund, Sweden.*

IRVING I. GESCHWIND, *Hormone Research Laboratory, University of California, Berkeley, California.*

BERTIL LINDQUIST. *Department of Pediatrics, University of Gothenburg Children's Hospital, Gothenburg, Sweden.*

MORTIMER B. LIPSETT, *National Cancer Institute, National Institutes of Health, Bethesda, Maryland.*

J. F. MANERY, *University of Toronto Faculty of Medicine, Toronto, Canada.*

HENRY R. MAHLER, *Department of Chemistry, Indiana University, Bloomington, Indiana.*

IRVING L. SCHWARTZ, *Medical Research Center, Brookhaven National Laboratory, Upton, New York.*

REIDAR F. SOGNNAES, *Harvard School of Dental Medicine, Boston, Massachusetts.*

NIELS A. THORN, *Institute of Medical Physiology, University of Copenhagen, Denmark.*

Preface

There comes a time in the development of any subject matter when a comprehensive treatment is both feasible and useful. It is over twenty years since Shohl published his classic text and thus defined the subject matter with which this treatise deals. Mineral metabolism was then in its infancy; much has been learned since, not only about behavior and function, but also about new methods and approaches. Indeed, these new concepts and techniques have proved so fruitful and give such promise for the future that soon the subject area will no longer be encompassed by two volumes and two editors, no matter how ably assisted by contributing authors.

This treatise reviews critically the dynamic and functional aspects of mineral metabolism — and thus will serve as a reference and text for research scientists and advanced students in biology, medicine, and agriculture whose interests touch upon the role of minerals in physiology, biochemistry, biophysics, or nutrition. We hope that this work will consolidate existing knowledge for the specialist and enable workers to adapt to their fields of interest new approaches developed in other areas.

Volume I (Part A, Chapters 1–10, and Part B, Chapters 11–16) treats of the physical and biological principles required for an analysis of the metabolism of minerals. Volume II will deal with the elements themselves.

The introductory chapter of Volume I gives a historical perspective, with homeostasis as a central theme. The next chapters deal with thermodynamics, cybernetics, chelation, compartmental analysis, and ion transport; by concise statement and illustrations they provide for the biologist the tools and framework of physical biology on which physiological analysis must rest. Chapter 7, Body Fluid Dynamics, characterizes the major systems and mechanisms involved in mineral movement. The succeeding three chapters deal with the processes concerned in mineral intake and output, namely, intestinal absorption and excretion, renal output, and extrarenal excretion. This completes Part A. Chapters 11 and 12 cover the hormonal regulation of mineral metabolism, while the next three chapters treat of the major tissues (connective, bones, and teeth) that are the principal reservoirs for minerals in the body. Chapter 16 interprets some of the enzymatic processes that are dependent on mineral and metal activation.

Traditionally mineral metabolism has dealt with the organism as a whole, rather than with the individual cell. We have followed this tradition, even though it has meant the omission of much that is fascinating and perhaps pertinent. This tradition also implies emphasis on the mammalian organism, particularly man.

Clearly no one person can write a treatise such as this, and we as editors are grateful to the contributors for the authoritativeness of their chapters. Coverage, overlapping discussions, and omissions are naturally the province and responsibility of the editors. We are therefore most appreciative of the contributors' willingness to provide two and often several versions of their chapters, as this procedure made it possible to achieve an integrated approach and some unity.

For critically reviewing manuscripts we wish to express our appreciation to: F. Brink, Jr., G. L. Brownell, C. A. M. Hogben, C. M. McCay, W. L. Nelson, L. C. Norris, T. Shedlovsky, R. C. Swan, and D. D. Thompson.

As always, there must be compromises between complete coverage and economics of book size. Some traditional subject matter such as hydrogen ion relationships had to be omitted in the realization that this information is available in other books. For the same reason, the historical approach was discouraged. Throughout, the material to be covered and the bibliographies are meant to be selective rather than exhaustive; however, where the subject matter is reviewed for the first time, the coverage is intended to be as complete as possible. Naturally, the emphasis has been on the newer developments. Because this book was nearly three years in the making, some chapters were obviously written much sooner than others. However, all authors have attempted to include at least the most important current references, even if they had to be added to the proofs.

Finally, we want to thank the Publisher and his staff for their patience and encouragement while this work was in progress.

C. L. Comar
Ithaca, New York

Felix Bronner
New York City

May, 1960

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Chapter 1

Introduction : Homeostasis in Mineral Metabolism

Franklin C. McLean

Mineral metabolism, with a subject matter of its own, dates from the publication by Klinke (1931) of *Der Mineralstoffwechsel : Physiologie und Pathologie*, which includes material selected from a wide variety of sources. In the same year Peters and Van Slyke (1931) published *Quantitative Clinical Chemistry : Volume I, Interpretations*, also drawing upon a large volume of material concerning the normal and pathologic physiology of the mineral elements, but without systematic reference to mineral metabolism as such. Eight years later Shohl (1939) published his *Mineral Metabolism*, which has long been the standard work of reference in the field. Since that date there have been many monographs in special areas of mineral metabolism and on collateral subjects, but up to this writing there appears to have been no further effort to treat the subject matter as a whole, and the comprehensive title has not again appeared.

The present volume affords an opportunity to compare the state of the subject of mineral metabolism of the 1960's with that of the 1930's. By 1939 the literature was already voluminous ; it has increased since then by geometric progression. Phosphorus metabolism, for example, treated by Shohl in 17 pages, has been the subject of two symposia comprising 1692 pages (McElroy and Glass, 1951, 1952).

But the changes over a period of 20 years cannot be described in terms of quantity alone. New concepts have entered the literature and demand increasing attention. The most striking are those which deal with the kinetics of the metabolism of the mineral elements, studied in relation to transport and by means of compartmental analysis, with homeostatic mechanisms, and with enzymatic activities barely envisaged twenty years ago. It is no longer possible to deal with this subject in static terms ; the dynamic approach is everywhere apparent in this volume and the second to follow.

Homeostatic mechanisms, of the topics just mentioned, are of primary and fundamental importance. This introductory chapter will consider the regulatory mechanisms concerned in mineral metabolism within a frame of reference that may be helpful in unifying and reviewing the individual chapters of this treatise. Volume I, Chapter 2 presents thermodynamic details that can serve as a basis for quantitative considerations. Volume I, Chapter 3 includes a full treatment of control system theory and information theory. It uses the control of blood glucose as an example of the application of physical theory to homeostasis in a complex biological system, but does not attempt to treat this aspect of mineral metabolism. Other chapters touch upon homeostatic mechanisms of the particular systems or elements with which they deal. Self-regulation of the hydrogen ion concentration of the blood, a subject closely related to the content of this book, has found its place in many monographs and in all standard texts of physiology and biochemistry and appears only incidentally in the present work.

The concept of self-regulation in biological systems was first introduced by Claude Bernard (1878), who made the distinction between the *milieu extérieur*, in which the whole organism exists, and the *milieu intérieur*, in which the cells live. After describing some of the mechanisms by which the relative constancy of the internal environment is maintained, he wrote: " . . . tous les mécanismes vitaux, quelque variés qu'ils soient, n'ont toujours qu'un but, celui de maintenir l'unité des conditions de la vie dans le milieu intérieur." In a statement frequently quoted, Haldane (1922) has said: " No more pregnant sentence has ever been framed by a physiologist."

This concept was further developed by Walter B. Cannon (1926, 1929, 1932). In 1929 he wrote: " The coordinated physiological reactions which maintain most of the steady states in the body are so complex, and so peculiar to the living organism, that it has been suggested (Cannon, 1926) that a specific designation for these states be employed—*homeostasis*." In recent years the growth of control and communications systems and theory has led to attempts to apply their principles to biology. This field, called " cybernetics " by Wiener (1948), from the Greek word for " steersman," has found particularly useful applications in the understanding and analyses of the processes employed by the organism to maintain the constancy of the conditions of life in the internal environment (cf. Vol. I, Chapters 2 and 3).

Claude Bernard, writing before the era of endocrinology, believed that the nervous system integrated the regulatory mechanisms responsible for the constancy of the composition of the internal environment. Cannon (1932) drew attention to the role of the autonomic nervous system, linking it with what he called the sympathico-adrenal system, his emphasis being largely on the function of the adrenal medulla in bringing about circulatory readjustments.

There is also an integrative action of the endocrine system, as described by Selye and Rosch (1954), who point out that the ability of man to adapt successfully to his environment depends mainly on the coordinative activities of the two great integrating systems—nervous and endocrine. The interplay between these systems is largely responsible for the regulation and control of the mineral elements in the internal environment, as well as for the other homeostatic mechanisms of which the organism makes use.

Cannon (1932) gave the name " fluid matrix " to the watery environment of the cells, which Claude Bernard had called the " internal environment." This includes the blood and all of the extracellular tissue fluids. It also includes the fluid, or semi-fluid, portions of the ground substance of connective tissue, which constitute a special portion of the extravascular fluids. As a broad generalization (cf. Vol. I, Chapter 13) it can be said that electrolyte concentrations in the fluids of connective tissue closely resemble those of an ultrafiltrate of plasma. Within the ground substance there are homeostatic adjustments between a colloid-rich, water-poor phase, on the one hand, and a colloid-poor, water-rich phase on the other, these being dependent upon changes in the state of aggregation of the colloids. Such changes may be induced by hormones ; an example is the influence of relaxin upon the state of aggregation in the connective tissue of the symphysis pubis (Engel, Joseph, and Catchpole, 1954).

Bone has a solid phase, consisting of collagen fibers and crystals of bone mineral, and a liquid phase, closely related both to the collagen and to the mineral. The interchange of mineral constituents between the solid phase and the fluids of the body is maintained in a steady state as a result of several processes—exchange of ions between the solid and the liquid phases, deposition of bone mineral (accretion), and its dissolution (resorption). The interactions between blood and bone, contributing to the homeostasis of both, are presented in detail in Vol. I, Chapter 14, which also describes the applications of compartmental analysis to the kinetics of the bone mineral (cf. Vol. I, Chapter 5).

The Acid-Base Balance of the Blood

The critical role of the respiratory center in the regulation and especially in the minute to minute control of the pH of the blood has long been appreciated. What has not been so well understood is the part played by chemical influences on the respiratory center, especially in relation to one another. Moreover, the roles of the chemoreceptors of the aortic and carotid bodies have come to be understood only recently. For some years there was a difference of opinion as to whether the pH or the carbon dioxide tension was the major chemical messenger affecting the activity of the respiratory center. Current belief is that there are three factors which influence the respiratory center, either by reflex action through the chemoreceptors, or by direct chemical effects on the center. These are oxygen tension, carbon dioxide tension, and hydrogen ion concentration. Through their combined effects, information on the state of the acid-base balance is transmitted to the respiratory center, in part indirectly through the chemoreceptors. This is an excellent example of the type of biological problem that can be studied by application of information theory (cf. Vol. I, Chapter 3) and demonstrates a feedback mechanism resulting in self-regulation of the pH of the blood.

Regulation of the pH of the blood is not, however, carried out solely by control of respiration. The kidneys have an equally important function in this respect but act more slowly. According to Cannon (1932): "The kidneys, more slowly adapting their functions to the situation, prevent accumulation of either non-volatile acid or alkaline salts," and his summation of the feedback mechanisms concerned with regulation of the pH of the blood is: "We may think of these various agencies [respiratory center; buffer action of the blood: kidneys] as sentinels, continuously on the alert, ready at the first indications of a change to act in such ways as to prevent a harmful swing away from that normal steady state of the blood which is near the neutral position."

The Kidneys and Homeostasis

The role of the kidneys in the regulation of the acid-base balance affords an opportunity to consider their part in the homeostatic regulation of the mineral constituents of the blood in general. Up until recent years it has been customary to regard the kidneys as functioning in an automatic fashion, with built-in mechanisms, responding to alterations in the composition of the blood plasma so as to conserve elements in short supply and to eliminate those in excess. A classic description of acid-base regulation by the kidneys, mainly from this point of view, is that of Pitts (1950) (cf. Vol. I, Chapter 9).

More recently it has become apparent that the ability of the kidneys to act automatically is limited, and that increasing importance must be ascribed to central and/or hormonal control. I am therefore advancing the thesis that the major cations of the blood plasma (Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+) are subject to such extrarenal control. In some cases the evidence is clear; in others it is only suggestive. On the whole, however, the generalization seems permissible, at least as a working hypothesis, that the concentrations of all of these cations in the blood are centrally or hormonally controlled, and the evidence is examined in this chapter.

It is further proposed that for regulation of the cations of the blood plasma, the organism is supplied with a number of information centers, of which the respiratory center is one; each of these responds to information as to a particular state of affairs. These centers then relay this information, through a " chain of command," to the organs whose function it is to apply the necessary corrections.

For the major anions of the blood plasma (Cl^-, HCO_3^-, $H_2PO_4^-/HPO_4^{2-}$, and SO_4^{2-}) the situation is quite different. As against the generalization that the cations of the blood are subject to central and/or hormonal regulation, the contrary statement may be advanced for the anions, that is, that the control of none has been shown to depend upon information transmitted from a higher center, and that the existence of a feedback mechanism has been found for none.

Homeostasis of the Cations of the Blood

With respect to homeostatic regulation of the cations, the major advance in recent years has been the discovery and partial elucidation of the control system which regulates the concentration of sodium in the body fluids (cf. Vol. I, Chapter 12). This system includes an extrahypophyseal diencephalic center, located in the hypothalamus, or perhaps, as more recently suggested (Farrell *et al.*, 1959), in the region of the pineal body. It also includes a glomerulotropic hormone, presumably formed by neurosecretory cells in the diencephalon, which controls the secretion of aldosterone by the zona glomerulosa of the adrenal cortex. Aldosterone then acts upon the tubular reabsorptive mechanism of the kidneys to regulate the retention of sodium in the organism.

This complex mechanism fulfills all of the criteria for an information center, coupled with a chain of command which eventually leads to the corrections that must be carried out by the kidney. This makes it abundantly clear that the kidney itself, while actually performing the functions that result in homeostasis, is far from doing so automatically, so far as sodium is concerned.

Aldosterone, the sodium-retaining hormone elaborated by the zona glomerulosa of the adrenal cortex, is a mineralocorticoid. Its properties are also exhibited by other adrenocorticoids, but aldosterone is many times more effective with respect to these properties than any other known natural steroid. The existence of a supraoptic information center which controls the output of aldosterone is well established (cf. Vol. I, Chapter 12), but many of the details of the control system remain in doubt. Farrell (1958) has proposed a description which, though certainly oversimplified, provides a working hypothesis as to how such a system might function. This system includes a regulatory center in the brain stem, responding primarily to the level of serum electrolytes, acting directly or by way of chemoreceptors. Its activity is also subject to alteration by higher centers, since it responds to emotional stimuli, such as anxiety and excitement. Stretch receptors, located in the atria and possibly in the great veins, respond to changes in the volume of body water, and exert a reflex effect upon the regulatory center.

It is believed (Gaunt and Chart, 1957) that neurosecretory cells of the regulatory center produce a tropic substance, called aldosteronotropin or glomerulotropin, which finds its way to the adrenal cortex, either directly by a humoral pathway or indirectly through the pituitary stalk and the adenohypophysis. This tropic substance in turn controls the output of aldosterone, which then influences the reabsorption of sodium by the renal tubules.

While this brief statement is satisfactory as a description of the functioning of the sodium-retaining system, and while the evidence is adequate to establish a central control system for the retention of sodium in the organism, it is deficient in many details. These matters are examined at length in Vol. I, Chapter 12.

The interrelationships between this system and that regulating urine volume by the antidiuretic hormone (ADH), also produced by neurosecretory cells in the hypothalamus, are not clear. It seems certain, however, that there is a reciprocal relationship between these mechanisms, whose homeostatic functions are so closely related.

The role of one of the factors concerned in regulation of the electrolyte composition of the plasma, i.e., the osmotic pressure of the blood, has been much clarified in recent years (Gaunt *et al.*, 1956), and this adds strength to the concept of a center which transmits information to the kidneys as the organs of excretion and conservation. In addition to the center in the hypothalamus, responding to changes in the concentration of electrolytes and/or to changes in the volume of the blood, there are osmoreceptors, sensitive to minute changes in effective osmotic pressure. The hypothalamus responds to increase in osmotic pressure by increasing its output of a hormone, known as vasopressin or as ADH. This also is believed to pass through the pituitary stalk, to the posterior lobe of the gland, and from there to be liberated into the blood stream. Its concentration in the blood plays a large part in the regulation of the reabsorption of water by the kidneys, mainly in the distal tubules. As already noted, ADH, while apparently under separate control, has an action reciprocal to that of the glomerulotropic hormone and aldosterone ; together they perform homeostatic regulation of the volume, osmotic pressure, and sodium and potassium concentrations of the blood plasma (cf. Vol. I, Chapter 7).

A satisfactory analysis of the relation of the sodium-retaining system to the regulation of the excretion of potassium and its concentration in the blood plasma is still lacking. There seems to be no doubt, however, that potassium is also under control of the same system, and it has even been proposed that the concentration of potassium in the blood plasma is a stimulus to the activity of the regulatory center (Laragh and Stoerk, 1957). Suffice it to say here that the regulatory mechanism influences also the behavior of potassium, but in a fashion reciprocal to its influence upon sodium (cf. Vol. I, Chapter 12). That sodium is the primary target of the regulatory system, with a secondary and indirect effect upon potassium, seems probable. The general principle of central and/or hormonal control for potassium is, however, valid (cf. Vol. II, Chapter 26).

As to magnesium, the situation is less satisfactory. In fact information concerning the metabolism and behavior of magnesium in the animal organism is in a generally unsatisfactory state (cf. Vol. I, Chapter 11*). Here it can only be said that there is evidence (DaVanzo *et al.*, 1958) that the excretion of magnesium is responsive to adrenocortical control and that aldosterone increases its excretion as it does that of potassium. Although the evidence is insufficient to establish central and/or hormonal control of magnesium excretion or of the level of magnesium in the blood, there seems to be no inconsistency with the generalization stated herein concerning cations.

Homeostasis of Calcium

The homeostasis of calcium constitutes a special case among the cations of the blood plasma. Whereas hormonal regulation of the calcium ion concentration in the blood mediated by parathyroid activity is well established, it appears that this is accomplished without participation of the central nervous system, the adenohypophysis, or the adrenal cortex. The existence of a parathyrotropic hormone, elaborated by the anterior lobe of the hypophysis, is no longer supported by any evidence, and the idea of such a direct link between the pituitary and the parathyroids has disappeared from modern works on endocrinology. Patt and Luckhardt (1942) were able to demonstrate, by perfusion of the parathyroid glands with serum depleted of calcium, that these glands respond directly to a lowered concentration of calcium in the plasma by increased activity. All of the current evidence supports this view.

Moreover, a part of the homeostatic control of the calcium ion concentration in the plasma is independent of the parathyroid glands, and is not known to depend on any other internal secretion, although vitamin D, in physiological amounts, is now regarded as contributing to this control (cf. Vol. I, Chapter 14). Together, vitamin D and the parathyroid hormone are the major factors in maintaining the plasma calcium concentration at the normal level of approximately 10 mg %. In the absence of the parathyroids the plasma calcium level falls to about 6–7 mg %

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* See also Vol. II, Chapter 22.

where it remains approximately constant, in spite of a high rate of exchange between bone and blood. It is believed that this level is maintained by diffusion from the labile stores of calcium in the bones, perhaps aided by the influence of vitamin D on the production of citrate in the bones, and the complexing of calcium by citrate (Neuman and Neuman, 1958).

Homeostasis of the Anions of the Blood

Contrary to the situation with respect to the cations, it has not been shown that the concentration of any anion in the blood (Cl^-, HCO_3^-, $H_2PO_4^-/HPO_4^{2-}$, or SO_4^{2-}) is subject to either central or hormonal control. Rather, the homeostatic control of the anions of the blood is carried out either automatically by the kidneys, or under the influence of cations for which specific central or hormonal regulatory mechanisms exist. In general, but not always, the excretion of Cl^- tends to parallel that of Na^+. Similarly, the excretion of phosphate ions is markedly influenced by parathyroid activity and by the level of calcium in the blood, although here again other influences may act to overcome this interdependency. All of the anions, of course, contribute to the effective osmotic activity of the plasma, certainly the most important factor in determining the excretion of water.

It has been suggested repeatedly that an increase in the concentration of phosphate in the plasma causes an increase in the activity of the parathyroid glands, associated with elaboration of a second hormone, with a specific effect on the excretion of phosphate in the urine. This would be consistent with the idea of a self-regulating mechanism for the concentration of phosphate in the blood. However, no one has as yet succeeded in isolating two hormones from the parathyroid glands, one calcemic and the other phosphaturic, nor has there been a conclusive demonstration of a direct effect of the level of plasma phosphate on parathyroid activity. In the absence of such proof, the influence of parathyroid extract upon phosphate excretion, first reported by Albright and Ellsworth (1929), has been attributed to a hemodynamic effect of the parathyroid hormone on the kidneys (cf. Vol. I, Chapter 11).

As to the other anions of the blood plasma, all of the available evidence points to the view that the chloride and bicarbonate ions are handled by the kidneys nonspecifically, and as indifferent electrolytes (cf. Vol. I, Chapter 9). As for phosphate and for glucose, total or almost total reabsorption occurs in the renal tubules as long as the maximum reabsorptive capacity T_m is not exceeded ; beyond this point excretion in the urine increases rapidly. The earlier concept of threshold substances has been replaced by that of T_m ; whatever the terminology, the kidney acts to conserve such substances, unless they are present in the blood in excess, and for Cl^- and HCO_3^-, this renal control is the only known homeostatic mechanism. The same considerations apply to SO_4^{2-}. With the possible exception of phosphate, the kidney appears to have the sole responsibility for regulating the concentration of anions in the blood plasma.

Conclusion

It is apparent, as it has been since Claude Bernard and Walter B. Cannon wrote on the subject, that homeostasis in the animal organism depends upon an integrated system, with free interplay between its constituent parts. There have been many advances in the understanding of the mechanisms participating in the maintenance of steady states in the mineral constituents of the fluids of the body, and they are recorded in detail in the separate chapters of these volumes.

On the other hand, important gaps remain to be filled. Central control of the function of the kidneys in the acid-base balance, while highly probable, requires further elucidation. Regulation of the magnesium content of the fluids of the body has been too little investigated, and the situation with respect to potassium is still unsatisfactory. As to the anions, the mechanism of control of the excretion of phosphate remains uncertain, and further study is needed.

In this chapter two generalizations have been proposed as working hypotheses. It seems likely that homeostatic control of the concentrations of the cations in the fluids of the body, and of their retention or excretion by the kidneys, is exerted largely by integration of neural and hormonal factors ; this proposal may be further investigated. In contrast with the cations, the anions which reach the glomerular filtrate in the kidneys appear to be dealt with in an automatic fashion by tubular reabsorption. Perhaps some unifying principle will be found here also.

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Chapter 2

Thermodynamic Principles and Concepts

Jay Boyd Best and John Z. Hearon

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I. Introduction

For purposes of study and discussion one can distinguish two fundamental aspects of the metabolic behavior of a tissue. A tissue or cell has intrinsic properties stemming from the physicochemical organization of its own constituents. A tissue is also subject to extrinsic regulation and has properties conferred upon it by neural and endocrine control systems because it usually forms a functional part of a coordinated multicellular organism. A distinction of this type is useful for both experimental and theoretical purposes. It seems reasonable to suppose that such a classification corresponds to the distinction made experimentally between *in vitro* and *in vivo* tissue properties. However, the theoretical framework most appropriate for the examination of each of these two aspects is different.

Conceptualizations like information transmission, theory of automata, and Nyquist stability theorems are relevant to the extrinsic regulation systems. These types of problems will not be dealt with here but will be discussed in Vol. I, Chapter 3. The present chapter will, on the other hand, be concerned with the limits set on the metabolic behavior of a cell or organ by its own physical constitution.

It is an article of faith among practically all contemporary students of metabolism that the properties exhibited by living tissues can ultimately be understood in terms of the same basic laws that govern the behavior of the physical world. As this notion has much to recommend it and as there is no reason to doubt its validity at the present time, it will be accepted in the present discussion without further apology.

The first and second laws of thermodynamics, the conservation of matter and the statistical-mechanical principle of microscopic reversibility, impose severe restrictions of remarkable generality upon the nature of conceivable processes consistent with physical and biological reality. It will be the purpose of this chapter to discuss the more relevant implications of these postulates and, with the help of a few supplementary phenomenological expressions, to give a theoretical framework for the examination and interpretation of metabolic processes. Such a framework serves to define the domain of metabolic activity of a cell. Extrinsic regulatory mechanisms may modulate this activity only within the limits of this domain. Thus, a pattern of impulses impinging on a muscle via motor nerve endings may regulate the amount of contraction of the muscle but cannot cause it to violate the first or second laws of thermodynamics. Historically, thermodynamics has been developed primarily around the needs and interests of the physical chemist; therefore many of the classical theorems require some modification and rederivation to be applicable to situations of biological interest. Several features seem to be outstanding characteristics of metabolic processes: (*1*) chemical reactions are catalyzed enzymatically; (*2*) the systems involved can usually exchange matter with their surroundings; (*3*) chemical reaction systems are frequently not spatially homogeneous, therefore spatial flows of matter and the geometry of the system are important; (*4*) coupling frequently occurs between different chemical reactions and between these reactions and other processes such as the transport of electric charge, mechanical work, etc.; (*5*) the steady state, rather than equilibrium, is frequently the simplest experimental situation attainable; (*6*) the number of consecutive reaction steps involved in most metabolic processes is large; (*7*) metabolic conversions in which there is a large change in free energy for the over-all process frequently tend to be carried out stepwise, with the individual steps involving small changes in free energy.

These characteristics are, of course, not all independent, e.g., (*6*) can be viewed as a consequence of (*7*). However, they may be used as guides to the types of thermodynamic systems and processes most apt to be useful in the investigation of biological phenomena.

II. The First and Second Laws of Thermodynamics

One can, at the outset, distinguish between two regions, the *system* of interest and the *environment*. The system may exchange heat or matter with the environment ; the system may do work upon or have work done upon it by the environment ; and the system may undergo changes in its internal composition or configuration. If a system can exchange neither heat, work, nor matter with its surroundings, then it is said to be *isolated*. If it cannot exchange matter with its surroundings, it is said to be *closed*. If it can exchange matter with its surroundings it is said to be *open*. A process in which no heat is exchanged with the environment is called *adiabatic* ; one in which no work is done, *isochoric* ; one in which the pressure is held constant, *isobaric* ; and one in which the temperature is held constant, *isothermal*.

The first law of thermodynamics is a statement of the conservation of energy and of the equivalence of heat and work. The usual statement of it for a closed system is that

$$dE = đQ - đW \tag{1}$$

where dE is the increase in internal energy of the system, $đQ$ the increment of heat absorbed from the environment by the system, and $đW$ the increment of work done by the system upon the environment.

The first law places no restriction upon the interconversion of the various forms of energy. On the other hand, the second law does place such a limitation upon the conversion of heat into work. There are a number of ways of stating this restriction, all of which can be demonstrated (cf. MacDougall, 1939 ; Fermi, 1937 ; Zemansky, 1943) to be completely equivalent.

One of the more useful statements of the second law for a closed system is that

$$TdS \geqslant đQ \tag{2}$$

where dS is the change in entropy and T the absolute temperature at which the increment of heat $đQ$ is absorbed. When the process is reversible the equality sign holds, and the inequality sign obtains when the process is irreversible. If a system is isolated, then it can exchange no heat with its environment and will of course be closed, so that

$$(dS)_{\text{isolated}} \geqslant 0 \tag{3}$$

From Eq. (3) it is apparent that the entropy of an isolated system can never decrease. An isolated system will therefore be in its most stable state when its entropy is a maximum.

It will be advisable to digress slightly at this point to clarify a few matters of importance. There will be some set of numbers necessary to characterize the system. For example, in the case of a homogeneous gas comprised of a single molecular species it will be sufficient to specify the total number of moles n, the temperature T, and the pressure P to specify completely the state of the system. Alternatively, for such a system, one might choose P, n and V, or V, T, and n, where V denotes the volume of the system. When values have been assigned to the variables necessary to define the state of the system, the system will be in a particular state. When the system is in a particular state, all of the state variables will have unique values. A number of quantities of interest in thermodynamics are exclusively functions of the variables of state. If a system is taken from one particular thermodynamically defined state to another, the difference in such a quantity will be independent of the process by which the transition was effected and be dependent exclusively on the initial and final states. The infinitesimal increment of change in such quantities has the mathematical properties of a perfect differential. Not all quantities of thermodynamic interest have this property. For example, the heat absorbed or the work done by a system in passing from one state to another *do* depend upon the path by which the transition was accomplished. One cannot, therefore, associate a unique value of such a quantity with each state of the system nor will their infinitesimal increment of change have the mathematical properties of a perfect differential. A notational device is used to distinguish between those changes that are perfect differentials and those that are not. Thus dE and dS are perfect differentials while $đQ$ and $đW$ are not.

The conditions under which the entropy change can be used as a criterion of spontaneity of a process are experimentally difficult to achieve and seldom encountered in the laboratory. Because of this it is useful to introduce a function F called the (Gibbs) free energy and defined by the relation

$$F = H - TS \tag{4}$$

where H, the enthalpy or heat content, is defined as

$$H = E + PV \tag{5}$$

and V and P are the volume and pressure. The work that a system does can be divided into pressure-volume work and nonpressure-volume work. Denote the increment of nonpressure-volume work as $đW'$. Then

$$đW = đW' + PdV \tag{6}$$

Consider a closed system, capable of doing nonpressure-volume work, undergoing an infinitesimal transition between two thermodynamically defined states at the same temperature and pressure. By differentiation of Eqs. (4) and (5), setting dP and dT equal to zero, and use of Eq. (2) one obtains† that

$$(dF)_{T,P} < dE + PdV - đQ \qquad \text{irreversible process}$$

$$(dF)_{T,P} = dE + PdV - đQ \qquad \text{reversible process}$$

and, from Eqs. (1) and (6), that

$$\begin{aligned} (dF)_{T,P} &< -đW' &\quad& \text{irreversible process} \\ (dF)_{T,P} &= -đW' && \text{reversible process} \end{aligned} \tag{7}$$

If the system is such that only pressure-volume work can be done by it or on it, then $đW' = 0$ and $(dF)_{T,P} \leqslant 0$. Any spontaneous process by such a system at constant temperature and pressure must therefore entail a decrease in its free energy. A system subject to these restrictions will thus achieve its most stable configuration when its free energy is a minimum. The free energy of a closed system at constant temperature and pressure can increase only by having nonpressure-volume work done upon it by the environment.

III. Work, Forces, and Potential Functions

In the preceding discussion there was some mention of work, pressure-volume work and nonpressure-volume work. It was assumed that the reader has some previous knowledge of the concept of work. In the usual thermodynamic treatments found in elementary physical chemistry texts, work other than pressure-volume work is frequently not considered. In the course of mineral metabolism by biological systems various forms of nonpressure-volume work play an important role. For example, the metabolic processes involving phosphorus and potassium in striated muscle are coupled to the contraction of the actomyosin polymers and thus involve length-tension work. The metabolic processes of the kidney are, in some fashion, coupled with the performance of osmotic work. Frog skin (discussed in Volume I, Chapter 6) and the electric organs of the eel do electrical work, and their metabolic transformations are associated with this work. Because of the variety of the processes to which the investigator in the field of mineral metabolism may conceivably apply the contents of this chapter it will be advisable to discuss the notion of work at greater length.

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† The variables held constant in a differential change or partial differentiation shall be indicated as subscripts to the parenthesis enclosing the differential.

Although work, when expressed in terms of length, mass, and time, has the same dimensions as any other form of energy, it is most simply conceived in terms of some sort of generalized force multiplied by its conjugate displacement. This idea of work thus leads to the notion of the driving force associated with a process. These notions can be clarified by a few examples.

A tension τ is applied to an elastic fiber that is stretched an increment of length dl. The work done by the fiber on the environment will then be $-\tau dl$. A surface film with surface tension γ is increased in area by an infinitesimal amount dA. The work done *by* the film *on* the environment is thus $-\gamma dA$. An amount of charge dq is moved from electric potential ψ_1 to electric potential ψ_2. If $\Delta\psi = \psi_2 - \psi_1$ then the work done *by* the system *on* the environment is $-\Delta\psi\, dq$. The length is thus the conjugate displacement to tension, surface area conjugate to surface tension, and charge transferred conjugate to electric potential difference.

Anticipating some of the subsequent discussion the following statement can be made. If L is a variable whose specification gives the extent to which some process, of which a closed system is capable, has occurred, then $-(\partial F/\partial L)_{T,P} = f$ can be regarded as the " driving force " exerted by the system to make the process occur at constant temperature and pressure. For additional discussion of nonpressure-volume work terms see Zemansky (1943).

Since the processes of a biological system are carried out almost entirely under conditions of temperature and pressure that are very nearly constant during the process, the free energy [because of Eq. (7)] is the most important of the various thermodynamic functions for the discussion of metabolism. It will therefore be appropriate to indicate some of its more important properties and its relationship to other thermodynamic variables.

Consider a system capable of doing an increment of nonpressure-volume work on the environment by exerting some type of generalized force f and undergoing an infinitesimal conjugate displacement dL. This system may be open or closed. The free energy F of the system will then be a function of T, P, L, and the chemical composition. Let us assume that the chemical composition can be specified† by giving the number of moles $n_1, \ldots, n_c$ of C independent components.

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† This will not be the case in a system comprised of different phases or in one in which the concentrations are not uniform, although the modifications required are straightforward and will be discussed later on. Nonisotropic systems such as strained elastic solids must also be excluded and will not be discussed in the present exposition.

It can be demonstrated that

$$\left[\frac{\partial F}{\partial T}\right]_{P,\,L,\,n_1,\,\ldots} = -S \qquad -\left[\frac{\partial F}{\partial L}\right]_{P,\,T,\,n_1\,\ldots} = f \qquad \left[\frac{\partial F}{\partial P}\right]_{T,\,L,\,n_1,\,\ldots} = V \tag{8}$$

for both open and closed systems.

In the usual chemical system the free energy is said to be an *extensive* thermodynamic variable. This means that F is a function of the mole numbers $n_1, \ldots, n_c$ in such a way that if they were altered to the values $\lambda n_1, \ldots, \lambda n_c$ then the free energy would be altered to the value λF. Mathematically speaking this means that F is a homogeneous function of degree one in the mole numbers and by Euler's theorem (cf. Courant, 1936, p. 109) can be written in the form

$$F = \mu_1 n_1 + \ldots + \mu_c n_c = \sum \mu_k n_k \tag{9}$$

where $\mu_1 = \left[\dfrac{\partial F}{\partial n_1}\right]_{P,\,T,\,L,\,n_2,\,\ldots}$, $\mu_2 = \left[\dfrac{\partial F}{\partial n_2}\right]_{T,\,P,\,L,\,n_1,\,n_3,\,\ldots}$, etc. (10)

Differentiating F and using the expressions in Eq. (8), one can write that

$$dF = \sum_k \mu_k dn_k - SdT + VdP - fdL \tag{11}$$

and by differentiating Eq. (9) one can write

$$dF = \sum_k \mu_k dn_k + \sum_k n_k d\mu_k \tag{12}$$

By comparing Eqs. (11) and (12) one obtains that

$$VdP - SdT - fdL = \sum_k n_k d\mu_k \tag{13}$$

Under circumstances of constant temperature and pressure, dP and dT are zero so

$$-fdL = \sum_k n_k d\mu_k \tag{14}$$

It should be noted that fdL is the increment of nonpressure-volume work reversibly performed by the system at constant temperature and pressure. For an *open* system in which the free energy is a homogeneous function of degree one of $n_1, \ldots, n_c$ then

$$(dF)_{T,P} \leqslant -\text{đ}W' + \sum_k \mu_k dn_k \tag{15}$$

where the inequality holds for irreversible processes and the equality for reversible ones. Equation (15) should be compared with Eq. (7) that was previously obtained for closed systems. In the event that $dW' = 0$ and the system is thermodynamically defined by $T, P, L, n_1, \ldots, n_c$ then

$$(dF)_{T,P,L} = \sum_k \mu_k dn_k \tag{16}$$

whether the process is reversible or irreversible.

Consider a closed system comprised of C constituents in p phases. Let us suppose these phases to be all at the same temperature and pressure and the state of the system to be completely specified by giving, in addition to P, T and L, the number of moles of each constituent in each phase. Let n_{kj} and μ_{kj} respectively denote the mole number and the chemical potential of the kth constituent in the jth phase. The free energy change for such a system, when no external nonpressure-volume work is done and the pressure and temperature are constant, is

$$(dF)_{T,P,L} = \sum_{k=1}^{C} \sum_{j=1}^{p} \mu_{kj} dn_{kj} \tag{17}$$

In most systems of interest, the changes dn_{kj} will not all be independent. Depending on the nature of the process under consideration there will be *equations of constraint* imposing various restrictions on the system by requiring that certain relationships between these changes in the mole numbers be fulfilled. Thus, for example, if the system is closed and the constituents do not undergo chemical transformation then

$$\sum_{j=1}^{p} dn_{kj} = 0 \qquad k = 1, \ldots, C \tag{18}$$

Imagine a process in which dn_1 moles of the 1st constituent are transferred from the rth to the mth phase but none of the other constituents are permitted to change. The equations of constraint will then be

$$dn_1 = -dn_{1r} = dn_{1m}$$

$$dn_{kj} = 0 \quad \text{for } k \neq 1, \quad j \neq r \text{ or } m$$

Imposing these restrictions on the system reduces Eq. (17) to the form

$$(dF)_{T,P,L} = (\mu_{1m} - \mu_{1r}) dn_1 \tag{19}$$

From Eq. (7) we know, however, for the process to occur it must be true that $(dF)_{T,P,L} < 0$. It is clear from Eq. (19) that this can be satisfied only if $\mu_{1r} > \mu_{1m}$. We thereby come to the important conclusion that in a system *in which temperature and pressure are constant, a substance can move spontaneously only from regions of higher chemical potentials to regions of lower chemical potentials.*

From the proposition just obtained, an analogy between the chemical potential and electric potential becomes apparent. The analogy is complete and one can, in fact, regard the chemical potential as a generalization of the notions of potential energy in classical mechanics and electrical potential in electrical theory. In a purely electrostatic system the transport of charge from a region of low to high potential does not occur except by the use of some " pumping " device such as a battery. Similarly, if we observe a transport of a material in a biological system from a region of low to high chemical potential, we are forced to infer the presence of some active transport mechanism (cf. Vol. I, Chapter 6).

In the case of ideal, dilute solutions it can be demonstrated (MacDougall, 1939, pp. 143–145 or Glasstone, 1946, p. 685) that the chemical potential μ_k may be written in the form

$$\mu_k = \mu_k^0 + RT \ln C_k \tag{20}$$

where R is the gas constant, C_k the molar concentration and μ_k^0 the so-called standard chemical potential of the kth substance. The μ_k^0 are functions of T, P, and the nature of the solvent. In a system with a number of different solvent phases, the standard chemical potential, and the molar concentration of the kth component in the jth phase can be designated respectively as μ_{kj}^0 and C_{kj}.

Consider a system comprised of two different solvents, e.g., water and oil, in contact with one another. If a solute is dissolved in such a system it will partition itself between the two solvent phases in a manner that depends upon its relative solubility in them. At equilibrium one might ask for the relation between the concentrations in the two phases. It has already been demonstrated that for equilibrium the chemical potentials of the solute in the two phases must be the same. Thus, requiring that $\mu_{k1} = \mu_{k2}$ and using Eq. (20) one finds that

$$\left[\frac{C_{k1}}{C_{k2}}\right]_{\text{equil}} = e^{\mu_{k2}^0 - \mu_{k1}^0 / RT} \tag{21}$$

One might, if one is of a dubious frame of mind, ask whether an expression such as Eq. (21) really contributes anything to the description of the partitioning of a material between solvent phases. Why not, one might ask, merely measure the partition coefficient for the solute between the phases and be done with it? Suppose there are n different solvents. With these n solvents one could have $n(n-1)/2$ distinct two-phase systems, so without Eq. (21) one would have to measure $n(n-1)/2$ partition coefficients for any given solute. The number of independent differences of the standard chemical potentials involved is however only $n-1$, so that with the aid of Eq. (21) one need only make $n-1$ measurements to determine the $n(n-1)/2$ partition coefficients. It is clear that such a relationship is not just a tautology providing merely another interpretation of the same phenomena, but has considerable predictive value.

Another example will be useful to demonstrate a relationship between the geometry of a system and the equations of constraint as well as to derive a point of interest for an aspect of mineral metabolism. In the ordinary fluid chemical system the only geometrical variable of significance is its volume. In some cases, however, such as a system with a fine granular structure, one must take into account not only the bulk phases but also the surface and interfacial phases. In general the amount of material present in the surface and interfacial phases will be related to that in the bulk phases by equations of constraint containing geometrical parameters other than the volume.

The mineral component of bone is comprised of minute crystals of calcium phosphate believed to have the crystalline lattice structure of hydroxyapatite (Neuman and Neuman, 1953). The area of contact of these crystals with the interstitial fluids of the body is very extensive, leading one to suspect that the thermodynamic contribution of the surface phase may be considerable.

Consider a crystal of surface to volume ratio g and volume V. Denote the thickness of the crystal surface phase as δ. Suppose that ν_C calcium ions and ν_P phosphate ions are contained in an elementary lattice unit of the crystal. Let $\bar{V}$ be the volume of a mole of such lattice units and assume, for purposes of the present treatment, that it is the same for both the surface and interior phases of the crystal. Denote the chemical potentials of the lattice units in the surface and interior phases respectively as μ_s and μ_i. Let n_i and n_s denote respectively the the number of moles of lattice units in the interior and surface phases of the crystal. If the crystal is in contact with a supernatant solution of calcium and phosphate ions, it can grow by a process in which these ions pass from the solution into the crystal. One can write a stoichiometric equation for this process similar to that for chemical reactions. Thus

$$\underset{\text{solution}}{\nu_C Ca^{2+}} + \underset{\text{solution}}{\nu_P\left(PO_4^{3-}\right)} \rightleftarrows \underset{\text{interior}}{\frac{n_i}{n} Ca_{\nu_C}(PO_4)_{\nu_P}} + \underset{\text{surface}}{\frac{n_s}{n} Ca_{\nu_C}(PO_4)_{\nu_P}}$$

where $n = n_i + n_s$. If n_C and n_P are respectively the number of moles of calcium and phosphate ions in solution then

$$\begin{aligned} dn_C &= -\nu_P dn \\ dn_P &= -\nu_P dn \\ dn_i &= (1 - g\delta) dn \\ dn_s &= g\delta dn \end{aligned} \tag{22}$$

can be written as the equations of constraint for the process. By use of Eqs. (22), (20), and (17) one finds that the free energy change is

$$\frac{dF}{dn} = -\mu^0 - RT \ln C_{\mathrm{C}}^{\nu_{\mathrm{C}}} C_{\mathrm{C}}^{\nu_{\mathrm{P}}} + \mu_i(1 - g\delta) + \mu_s g\delta \tag{23}$$

where C_{C}, C_{P}, μ_{C}^0 and μ_{P}^0 are respectively the molar concentrations and the standard free energies in solution of calcium and phosphate ions and $\mu^0 = \nu_{\mathrm{C}}\mu_{\mathrm{C}}^0 + \nu_{\mathrm{P}}\mu_{\mathrm{P}}^0$. The system will be in equilibrium when $dF/dn = 0$, hence

$$\left[C_{\mathrm{C}}^{\nu_{\mathrm{C}}} C_{\mathrm{P}}^{\nu_{\mathrm{P}}}\right]_{\mathrm{equil}} = e^{(\mu^0 - \mu_i)/RT} \quad e^{[(\mu_i - \mu_s)/RT]g\delta} \tag{24}$$

Define

$$K = e^{(\mu^0 - \mu_i)/RT} \quad e^{[(\mu_i - \mu_s)/RT]g\delta} \tag{25}$$

$$K_0 = e^{(\mu^0 - \mu_i)/RT} \tag{26}$$

It will be noted that Eq. (24) is the so-called solubility product relationship for a solution of calcium and phosphate ions in contact with the crystalline phase of these ions. If the crystals are large and regular, then g will be effectively zero, so the value of the solubility product will be K_0. The quantity K_0 is independent of g and so will be the same for crystals having the same elementary lattice structure and composition, provided only that they are sufficiently large. The quantity K will not be independent of the geometrical parameter g, however, and will be different for crystals of different surface to volume ratio even though they are identical in other respects.

Since the value of g almost certainly is not the same for the various crystals in a sample of bone salt, having instead some type of frequency distribution, there will not exist any unique value of K for the sample. This will mean that there exists no product of the form $C_{\mathrm{C}}^{\nu_{\mathrm{C}}}\ C_{\mathrm{P}}^{\nu_{\mathrm{P}}}$ such that the solution of calcium and phosphate ions can be in equilibrium with all the crystals of a sample of bone salt. One is thereby led to conclude that no solubility product exists for the bone salt. This is in agreement with the comments of Hodge (1951) and Neuman and Neuman (1953) who point out that no one has yet observed a solubility product for bone crystals in contact with a calcium phosphate solution.

IV. Chemical Reactions and Affinity

Let us now examine the situation when the constituents of a system are capable of participating in chemical reactions. Consider the chemical reaction

$$m_1B_1 + m_2B_2 + \dots \rightleftarrows m_iB_i + m_{i+1}B_{i+1} + \dots$$

in which m_1 moles of B_1, m_2 moles of B_2, etc. react stoichiometrically to give m_1 moles of B_1, m_{i+1} moles of B_{i+1}, etc. The convention shall be adopted that the m's are assigned *negative* values for the *reactants* and positive values for products.

If the system has only a single phase and the amount of any constituent can change only by its participation in the reaction, then the equations of constraint for these participants can be written in the form

$$dn_k = m_k d\xi \tag{27}$$

where ξ is the " degree of advancement of the reaction " (deDonder and Van Rysselberghe, 1936) and dn_k the number of moles of substance *k produced* when the reaction proceeds by the amount $d\xi$. From Eqs. (16) and (27) the differential change in the free energy of the system with respect to ξ is

$$\frac{dF}{d\xi} = \sum m_k \mu_k \tag{28}$$

If, in addition, the reactants and products behave as perfect solutes, then by use of Eq. (20), Eq. (28) can be written as

$$\frac{dF}{d\xi} = \sum_k m_k \mu_k^0 + RT \sum_k m_k \ln C_k$$

The quantity $-dF/d\xi$ is called the " affinity " (deDonder and Van Rysselberghe, 1936) of the reaction.

Let the affinity of a reaction be denoted as α and the rate $d\xi/dt$, as u. If the extent of the reaction is *independent* of that of any other reaction in the system then one can assert from Eqs. (7) and (28) that

$$\alpha u \geqslant 0.$$

The reaction will be independent if it is possible to effect a virtual change in ξ without an obligatory virtual change in the extents of any of the other reactions. Conversely, if one finds for some over-all reaction process, denoted arbitrarily as Reaction 1, that

$$\alpha_1 u_1 < 0$$

then it can be inferred that this reaction is not independent but is *coupled* with some other reaction, which can be denoted as Reaction 2, such that

$$\alpha_1 u_1 + \alpha_2 u_2 > 0$$

If coupling between chemical reactions is considered to occur by chemical reaction, then a situation such as that presented by Reaction 1 would not occur if the complete, rather than just the partial, stoichiometry of the reaction were considered. For example the over-all reaction description

$$\text{adenosine diphosphate} + \text{inorganic phosphate} \rightarrow \text{adenosine triphosphate}$$

could be considered an example of Reaction 1 for the range of concentrations found biologically. This process is " coupled " with the over-all reaction involving the formation of lactic acid from glucose which could be considered analogous to Reaction 2. It should be noted, however, that the stoichiometry of the adenosine triphosphate synthesis reaction is incomplete, since a change in ξ_1 entails an obligatory change in ξ_2. In these remarks it has been assumed that $đW' \geqslant 0$.

If many reactions are proceeding, as is usually the case in practice, then the preceding discussion can be extended as follows. Let ξ_j be the degree of advancement of the jth reaction and m_{kj} the stoichiometric coefficient of the kth substance in the jth reaction. Then

$$dn_k = \sum_j m_{kj} d\xi_j \tag{29}$$

For the differential change in total free energy one therefore can write

$$dF = \sum_k \sum_j \mu_k m_{kj} \, d\xi_j \tag{30}$$

In this case the composition of the system will be completely specified by giving the initial number of moles of the various constituents and the degrees of advancement $\xi_1, \ldots, \xi_j, \ldots$ of the different reactions. The affinity, α_j, of the jth reaction will be $\sum_k \mu_k m_{kj}$.

From Eqs. (28) and (30) one can write the time rate of change of the free energy as

$$\frac{dF}{dt} = \sum_k \mu_k m_k \frac{d\xi}{dt} \tag{31}$$

for a closed system with a single reaction, or

$$\frac{dF}{dt} = \sum_k \sum_j \mu_k m_{kj} \frac{d\xi_j}{dt} \tag{32}$$

for a closed system in which a number of reactions are proceeding simultaneously.

Although the results embodied in Eqs. (28), (30), (31), and (32) apply only to closed systems, the extension of them to open and steady-state systems is straightforward. In these equations it was assumed that the change in the amount of any constituent is related by appropriate stoichiometric coefficients to the degrees of advancement of the reactions in which it is a participant. Let $d(n)$ be the differential increment of the number of moles of the kth constituent due to addition to the system from the environment. One can therefore assert, for an *open* system with many reactions of the type to which Eqs. (29) and (30) apply, that

$$dn_k = \sum_j m_{kj} d\xi_j + d_e n_k \tag{33}$$

The differential change in the free energy of such a system will therefore be

$$dF = \sum_k \sum_j \mu_k m_{kj} d\xi_j + \sum_k \mu_k d_e n_k \tag{34}$$

and the time rate of change

$$\frac{dF}{dt} = \sum_k \sum_j \mu_k m_{kj} \frac{d\xi_j}{dt} + \sum_k \mu_k \frac{d_e n_k}{dt} \tag{35}$$

where $d_e n_k/dt$ will be the *rate* of addition of the kth constituent from the environment to the system.

A *steady-state open* system may have reactions proceeding inside it, but its composition remains unchanged with time. The free energy of such a system will remain constant in time, but the free energy of the environment will decrease due to processes taking place in the system. In order for the composition to remain unchanged with time, the rate of addition of any constituent from the environment to the system must, of necessity, be equal to its net rate of consumption by the reactions in the system. Thus, in the case of a homogeneous system, to which Eq. (35) would be applicable, one would find, *under steady-state conditions*

$$\left[\frac{dF}{dt}\right]_{\text{system}} = 0 \tag{36}$$

$$\left[\frac{dF}{dt}\right]_{\text{environment}} \leqslant \sum_k \sum_j \mu_k m_{kj} \frac{d\xi_j}{dt} < 0 \tag{37}$$

$$\frac{d_e n_k}{dt} = -\sum_j m_{kj} \frac{d\xi_j}{dt} \tag{38}$$

In Eq. (37) it will be noted that a " less than or equal to " sign is used. Because it was assumed in the introduction of Eq. (30) that the composition of the system is spatially homogeneous, consideration of free energy dissipation due to concentration gradients within the system is excluded. Nothing was said, however, of concentration gradients in the surrounding medium or concentration differences across the enclosing surface of the system. Furthermore, the environment may be involved in unspecified irreversible processes having nothing to do with the exchanges within the system. All these will involve a dissipation of free energy over and above that occurring within the system.

V. Free Energy Dissipation and Spatial Flows of Matter

If the resistance to diffusion of a metabolite into or out of a cell occurs primarily at the cell surface, then there may be no appreciable concentration gradients in either the interior of the cell or the surrounding medium, aside from a " concentration drop " across the surface membrane. The maintenance of this concentration difference across the membrane requires a dissipation of free energy (cf. Vol. I, Chapter 6). If μ' and μ are the chemical potentials of the substance just outside and inside the membrane respectively, A the area of the membrane, and J the flow component (per unit time and area) into the cell perpendicular to the membrane, then this rate of dissipation at the membrane can be written

$$\left[\frac{dF}{dt}\right]_{\text{membrane}} = AJ(\mu - \mu') \tag{39}$$

for a single metabolite. If the material behaves like a perfect solute and the standard chemical potentials are the same on the two sides of the membrane, then Eq. (39) can be written (cf. Hearon, 1951) as

$$\left[\frac{dF}{dt}\right]_{\text{membrane}} = AJRT \ln \frac{C_i}{C_0} \tag{40}$$

where C_0 and C_i are the concentrations outside and inside respectively.

If the system under discussion is not spatially uniform, then the foregoing discussion needs some modification. Although the chemical potential of the kth substance may not be defined for the system as a whole, it will, except under unusual circumstances,† be defined for infinitesimal elements of volume of the system. The value of μ_k is, as we have seen from Eq. (20) for perfect solutes, a function of the concentration C_k and the nature of the solvent. In other cases it may also be a function of the total ionic strength [see discussion of the Debye-Huckel theory of the activity coefficient in Glasstone (1946) or MacDougall (1939)] or the electric potential at the point in question. Thus, in general, in a biological system one can anticipate that μ_k will have a unique value at a given point in the system at a given time, but may be different at different points in the system. Under these circumstances the total free energy is now given (Hearon, 1951) by

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† Such exceptional circumstances would occur in flame propagation, explosive reactions, and perhaps in the extremely rarefied gases of the upper atmosphere and outer space, but seem unlikely in systems of biological interest.

$$F = \int_V \sum \mu_k C_k dV \tag{41}$$

where μ_k and C_k are functions of the spatial coordinates of the system and the integral is extended over the volume V of the entire system. Equation (41) should be compared with Eq. (9) to which it reduces under the circumstances for which the latter is valid.

In general the composition, concentration gradients, electrical potentials, and chemical potentials within a living system will depend upon the rate of the metabolic processes taking place in the system. If the system is in the steady state, the spatial distributions of each μ_k and C_k in the system, although dependent upon these rates, remain invariant in time. Since the value of F for the system, whether given by Eq. (9) or (41), depends exclusively upon the spatial distributions of μ_k and C_k in the system, it will not change with time when the system is in steady state. Nevertheless, because the time-independent distributions of μ_k and C_k, determining the value of F, depend upon the rates, F will depend upon the rates. In this example we see an important interplay between " thermodynamics proper " and the kinetic considerations of rate (Hearon, 1951).

It will expedite the discussion considerably to introduce two mathematical notions used very routinely in physics but with which the average biological scientist may have no previous experience. Familiarity with the notion of a three-dimensional vector will be assumed.

The Inner Product. Denote by **i**, **j**, and **k** the three vectors of unit length directed in the positive directions of the x, y, and z axes respectively. Consider two vectors

$$\mathbf{u} = u_x\mathbf{i} + u_y\mathbf{j} + u_z\mathbf{k}$$
$$\mathbf{v} = v_x\mathbf{i} + v_y\mathbf{j} + v_z\mathbf{k}$$

whose respective components along the x, y, and z axes are u_x, u_y, u_z and v_x, v_y, v_z. The inner product, $\mathbf{u} \cdot \mathbf{v}$, (sometimes called the " dot product ") of **u** and **v** is a scalar quantity defined as

$$\mathbf{u} \cdot \mathbf{v} = u_x v_x + u_y v_y + u_z v_z$$

It can be demonstrated that if θ is the angle formed by the two vectors then

$$\mathbf{u} \cdot \mathbf{v} = uv \cos \theta$$

where u and v are the magnitudes of **u** and **v** respectively. According to these definitions it is interesting to note that

$$\begin{array}{ll} \mathbf{i} \cdot \mathbf{i} = 1 & \mathbf{j} \cdot \mathbf{k} = 0 \\ \mathbf{i} \cdot \mathbf{j} = 0 & \mathbf{j} \cdot \mathbf{j} = 1 \\ \mathbf{i} \cdot \mathbf{k} = 0 & \mathbf{k} \cdot \mathbf{k} = 1 \end{array}$$

The Gradient. Consider a differentiable scalar function ϕ of the spatial coordinates x, y, and z. The mathematical symbol $\nabla\phi$ is called the *gradient* of ϕ. The gradient of ϕ is defined as

$$\nabla\phi = \mathbf{i}\frac{\partial\phi}{\partial x} + \mathbf{j}\frac{\partial\phi}{\partial y} + \mathbf{k}\frac{\partial\phi}{\partial z} \qquad (42)$$

The quantity $\nabla\phi$ is a vector the meaning of which can be understood in the following way. Consider an arbitrary point in three-dimensional space. At that point ϕ will have some unique value. If one moves a small distance dr away from that point in some arbitrary direction then ϕ will change a small amount $d\phi$. The rate of change of ϕ with distance, i.e., $d\phi/dr$, will obviously depend upon the direction chosen. There will be one direction, however, for which $d\phi/dr$ is greatest. The quantity $\nabla\phi$, at the point in question, will be a vector oriented in the direction for which $d\phi/dr$ is a maximum and having the magnitude of this maximum value of $d\phi/dr$. The quantity $-\nabla\phi$ will be a vector equal in magnitude and oppositely directed to $\nabla\phi$. It should be noted that if $d\mathbf{r} = \mathbf{i}dx + \mathbf{j}dy + \mathbf{k}dz$ is an infinitesimal vector displacement in three-dimensional space then

$$\nabla\phi \cdot d\mathbf{r} = \frac{\partial\phi}{\partial x}dx + \frac{\partial\phi}{\partial y}dy + \frac{\partial\phi}{\partial z}dz - d\phi$$

is the differential change in ϕ associated with $d\mathbf{r}$. If $\phi(1)$ and $\phi(2)$ are the values of ϕ at the points 1 and 2 respectively then

$$\phi(2) - \phi(1) = \int_1^2 \nabla\phi \cdot d\mathbf{r}$$

For further discussion of the gradient and related matters the reader is referred to Phillips (1933).

Aside from discontinuities at phase interfaces and membranes of infinitesimal thickness, the chemical potential of a substance will be a differentiable function of the spatial coordinates at any given time. The gradient of the chemical potential will therefore be defined throughout such bulk phase regions and the free energy change rate per unit volume due to the flux **J** of the substance will be $\nabla\mu \cdot \mathbf{J}$.

To clarify the notion of the flux of a quantity the following consideration may be helpful. If some quantity, e.g., heat, electric charge, matter, is flowing in a spatial region then at each point of the region, at a given time, the flow will have a direction. Imagine a small element of surface A perpendicular to the direction of flow at a point and containing the point. Denote by r_A the amount of the quantity flowing through this element of surface per unit time. The flux **J** of the quantity at the point in question will be a vector having the direction of the flow and magnitude r_A/A.

In general the time rate of change of the free energy of a spatially distributed system at constant temperature and pressure can be written formally (cf. Hearon, 1950a) as:

$$\frac{dF}{dt} = \left[\frac{dF}{dt}\right]_{\substack{\text{bulk phase}\\ \text{transport}}} + \left[\frac{dF}{dt}\right]_{\substack{\text{chemical}\\ \text{reactions}}} + \left[\frac{dF}{dt}\right]_{\substack{\text{entry and}\\ \text{exit of matter}}} + \left[\frac{dF}{dt}\right]_{\substack{\text{interfaces and}\\ \text{membranes}}} \tag{43}$$

where

$$\left[\frac{dF}{dt}\right]_{\substack{\text{bulk phase}\\ \text{transport}}} = \sum_k \int_V \nabla\mu_k \cdot \mathbf{J}_k dV \tag{44}$$

$$\left[\frac{dF}{dt}\right]_{\substack{\text{chemical}\\ \text{reactions}}} = \sum_k \int_V \mu_k q_k dV \tag{45}$$

$$\left[\frac{dF}{dt}\right]_{\substack{\text{entry and}\\ \text{exit of matter}}} = \sum_k \int_{A_b} J_{kA}\mu_{kA} dA \tag{46}$$

$$\left[\frac{dF}{dt}\right]_{\substack{\text{interfaces and}\\ \text{membranes}}} = \sum_k \int_{A_i} J_{kA}(\mu_{kA} - \mu'_{kA}) dA \tag{47}$$

The quantities $\mathbf{J}_k$, μ_k, and q_k are respectively the flux, chemical potential, and rate of production per unit volume of the kth constituent. In Eq. (46) the quantity J_{kA} is the flux of the kth constituent into the system through the element of system boundary surface dA and μ_{kA} is the chemical potential of the kth constituent at a point just interior to the surface. The integrals of Eqs. (44) and (45) are taken over the entire volume, i.e., they are the sum of the enclosed quantity for all the infinitesimal volume elements of the system. The integral in Eq. (46) is taken over the entire surface bounding the system, i.e., it is the sum of the enclosed quantity for all the infinitesimal surface elements of the surface A_b enclosing the system. The integral of Eq. (47) is also a surface integral but, instead of referring to the bounding surface, it refers to all those surfaces A_i of discontinuity enclosed within the system. For example, if the system under consideration is a metabolizing cell, the fourth integral would refer to all the internal membranes, such as the nuclear membrane and those enclosing the mitochondria and chloroplasts. In the case of such an internal membrane, J_{kA} is the flux through the element of surface dA, μ_{kA} the chemical potential immediately adjacent to dA on the side *from* which the positive direction of the flux is directed. If the bounding surface of the system is taken outside the cell so as to contain the cell membrane, then the integral of Eq. (47) will also be integrated over the cell surface with the positive direction for the flux being from the medium into the cell.

Equation (44) gives the free energy change rate due to flows of matter in the bulk phase regions of a system e.g. the cytoplasm of a cell, Eq. (45) gives that due to the production or consumption, i.e., chemical transformation, of the various constituents ; while Eq. (46) gives that arising from flows of matter, e.g., metabolites, across the enclosing surface into or out of the system. Equation (47) gives the rate of dissipation of free energy required to maintain the concentration differences across the various membranes and interfaces within the system. With the exception of the relation given in Eq. (47), Eq. (43) is essentially that derived by Hearon (1950a). It should be noted that if $J_{kA}(\mu_{kA} - \mu'_{kA})$ is constant over an entire surface, then Eq. (47), for a given metabolite, becomes the same as Eq. (39).

Closely associated with the notion of a flow or flux of matter is that of the " driving force " or " cause " of the flux. It was demonstrated in Section III that the relation between the chemical potential and spatial transport of matter is analogous to the relation between the electrical potential and spatial transport of charge.

If $\mathbf{v}_k$ is the average velocity (a vector) of the molecules of the kth constituent at some point then the flux at that point will be

$$\mathbf{J}_k = C_k \mathbf{v}_k \tag{48}$$

If Ω_k is the mobility of the kth constituent then $1/\Omega_k$ can be looked upon as a frictional coefficient for a mole of the kth constituent moving relative to the medium. Suppose that $\mathbf{X}_k$ is the force on a mole of the kth constituent. One might then write

$$\mathbf{v}_k = \Omega_k \mathbf{X}_k \tag{49}$$

on the common-sense supposition that the velocity of the kth constituent is proportional to the force driving it through the medium. To clarify these notions somewhat, it might be useful to consider the following. If N_A is Avogadro's number, then the average force on a single molecule will be $\mathbf{X}_k/N_A$. One can look upon $1/\Omega_k N_A$ as the magnitude of the viscous drag force exerted by the medium on a molecule of the kth constituent moving through it with unit velocity.

In keeping with the suggestion of Guggenheim (1929) and the previously discussed properties of the chemical potential, the force on a mole of the kth constituent at a point can be written

$$\mathbf{X}_k = -\nabla\mu_k \tag{50}$$

From Eqs. (48), (49), and (50) one can write the flux of the kth constituent in the form

$$\mathbf{J}_k = -\Omega_k C_k \nabla\mu_k \tag{51}$$

In the event that the solution is perfect (see Eq. 20) and the standard chemical potential μ_k^0 is spatially uniform,

$$\nabla\mu_k = \nabla(\mu_k^0 + RT \ln C_k) = \frac{RT}{C_k}\nabla C_k \tag{52}$$

If Eq. (52) is substituted into Eq. (51) and $\Omega_k RT$ is identified with the diffusion coefficient D_k one obtains the familiar relation

$$\mathbf{J}_k = -D_k\nabla C_k \tag{53}$$

known as Fick's law.

Since Eq. (51) reduces to Eq. (53) under the circumstances for which Fick's law is valid but does not contain these restrictions on its validity, it is obvious that it is far more general than Fick's law. For example, the constituent may be charged as in the case of an electrolyte and an electric field imposed on the system. Denote the chemical potential in the absence of the field as μ_i, the electric potential as Ψ, the Faraday constant (the charge of one equivalent of ions) as Γ, the valence of the kth species as z_k and the total electrochemical potential as $\bar{\mu}_i$. One can then write (MacDougall, 1939, p. 346) the total electrochemical potential as

$$\bar{\mu}_k = \mu_k + z_k\Gamma\Psi \tag{54}$$

If, as before, one assumes $\nabla\mu_k^0 = 0$ then one can write

$$\mathbf{J}_k = -D_k\nabla C_k - C_k\frac{z_k D_k \Gamma}{RT}\nabla\Psi \tag{55}$$

which, if one notes that the electric field intensity $\epsilon = -\nabla\Psi$, will be recognized as the expression used to describe electrophoretic migration. To obtain the stationary distribution of such a constituent in a one-dimensional system along the x axis, with field intensity $\epsilon = -\partial\Psi/\partial x$, one can set $\mathbf{J}_k = 0$ and Eq. (55) reduces to

$$\frac{1}{C_k}\frac{dC_k}{dx} = -\frac{z_k\Gamma}{RT}\frac{d\Psi}{dx} \tag{56}$$

Equation (56) can be integrated to obtain the equilibrium relation between the concentrations C_k' and C_k'' at any two potential values Ψ' and Ψ'' respectively. Thus

$$\ln\frac{C_k'}{C_k''} = -\frac{z_k\Gamma}{RT}(\Psi' - \Psi'') \tag{57}$$

It is also interesting to consider the case of a nonelectrolyte in a solvent whose properties are not spatially uniform. For example one might have a gradient in the composition of the solvent due to a gradient of some solute (other than that on which our attention is focused) present in large concentrations. If this is the situation, but Eq. (20) still is valid in any particular infinitesimal region, then, instead of Fick's law, one would obtain

$$\mathbf{J}_k = -D_k\nabla C_k - \frac{D_kC_k}{RT}\nabla\mu_k^0 \tag{58}$$

A comparison of Eqs. (58) and (53) will serve to show that in such situations it is as though a force of $-\nabla\mu_k^0$ were acting upon the solute in question. Consideration of the stationary distribution by setting $\mathbf{J}_k = 0$ in Eq. (58) and integration leads one to a result that is precisely the same as Eq. (21) for the partition between two discrete dissimilar phases.

It will be useful at this point to introduce another mathematical step routinely used in mathematical physics. Consider a vector $\mathbf{u}$. Suppose that each of the components u_x, u_y, and u_z is a differentiable function of the spatial coordinates x, y, and z. The *divergence* of $\mathbf{u}$, denoted as $\nabla\cdot\mathbf{u}$, is a scalar quantity defined as

$$\nabla\cdot\mathbf{u} = \frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} + \frac{\partial u_z}{\partial z} \tag{59}$$

The physical meaning of the divergence can be viewed in the following way. Consider some arbitrary spatial point in a system in which a flow of matter occurs. Imagine a small element of volume dV containing the point in question. Suppose that one examines the flux $\mathbf{J}_k$ of some given constituent at each point of the closed surface circumscribing the small element of volume. In this way one could ascertain the net rate of entry or exodus of the constituent for the volume element. One can say that $\nabla \cdot \mathbf{J}_k dV$ will be the net rate of exodus of the kth constituent from the volume element dV. From this it is apparent that $-\nabla \cdot \mathbf{J}_k dV$ is the net rate of entry of the kth constituent into the volume element.

If the kth constituent is neither produced nor destroyed within the element of volume dV, then $-\nabla \cdot \mathbf{J}_k dV$ will be the rate at which the kth constituent is accumulating or " piling up " within dV. The rate at which the kth constituent is accumulating within dV can also be written as $(\partial C_k/\partial t)dV$ where t is the time. From this one can assert that for any region in which the kth constituent is being neither created nor destroyed,

$$\frac{\partial C_k}{\partial t} = -\nabla \cdot \mathbf{J}_k \tag{60}$$

If q_k is the rate per unit volume at which the kth constituent is being produced in some region then in that region

$$\frac{\partial C_k}{\partial t} = -\nabla \cdot \mathbf{J}_k + q_k \tag{61}$$

It will be useful in the subsequent discussion to point out that, since the gradient of a function is a perfectly well-behaved vector, one can take the divergence of the gradient of a function. Thus

$$\nabla \cdot \nabla\phi = \nabla^2\phi = \frac{\partial^2\phi}{\partial x^2} + \frac{\partial^2\phi}{\partial y^2} + \frac{\partial^2\phi}{\partial z^2} \tag{62}$$

As an illustrative example let us take Eq. (53) for $\mathbf{J}_k$ and substitute it into Eq. (60) and assume D_k to be a constant. Doing this one obtains that

$$\frac{\partial C_k}{\partial t} = D_k \nabla^2 C_k \tag{63}$$

In the event that the concentration varies along the direction of the x axis but not along the directions of the y and z axes, one obtains

$$\frac{\partial C_k}{\partial t} = D_k \frac{\partial^2 C_k}{\partial x^2} \tag{64}$$

which will be recognized as the alternative statement of Fick's law frequently used in experimental studies (e.g., see Bull, 1943) involving the diffusion of a material in a one-dimensional system.

One of the fundamental requirements for a system to be in a steady state is that the concentrations of each of the constituents must be independent of the time, i.e., $\partial C_k/\partial t=0$ for all points in the system for every k. From Eqs. (60) and (61) it is apparent that when the system is in a steady state, then $\nabla \cdot \mathbf{J}_k$ must be zero in any region where the kth constituent is not being produced or consumed and $\nabla \cdot \mathbf{J}_k=q_k$ in any region where it is.

One can write an equation very similar to Eq. (60) expressing the conservation of electric charge in the system. Thus, if **I** is the intensity of the electric current, i.e., the flux of charge, at a point in a volume conductor and ρ is the net charge per unit volume, i.e., the space charge, then

$$\frac{\partial \rho}{\partial t}=-\nabla \cdot \mathbf{I} \tag{65}$$

In a system, e.g., a frog skin, through which an electric current is flowing there must be no time rate of change of the space charge distribution when the system is in a steady state. Thus, under steady-state conditions, $\partial\rho/\partial t=0$ which, because of Eq. (65), implies that $\nabla \cdot \mathbf{I}=0$.

Because, in a biological system, the transport of electric charge always occurs by migration of ionic constituents, Eq. (65) does not really represent any new condition in addition to those already stipulated for the steady state. To see this, one can note that

$$\mathbf{I}=\Gamma\sum_k z_k\mathbf{J}_k \tag{66}$$

and that

$$\nabla \cdot \mathbf{I}=\Gamma\sum_k z_k\nabla \cdot \mathbf{J}_k \tag{67}$$

Since in the steady state $\nabla \cdot \mathbf{J}_k=0$ for any ionic constituent that is neither created nor destroyed and the net change produced from chemical reaction must be zero, it is evident that $\nabla \cdot \mathbf{I}=0$.

Up to this point no mention has been made of convective flow or transport due to convection. The convective flow or bulk velocity shall be denoted as **v** (without any subscript) and have the units of volume per unit area per unit time. In discussing the fluxes of the various constituents it has been tacitly assumed that either the convective velocity is zero or that the reference frame to which the fluxes are referred is moving along with the fluid at the same rate as the convective velocity. A distinction shall now be made between the flux

$\mathbf{J}'_k$ relative to a fixed frame of reference and the flux $\mathbf{J}_k$ relative to a frame of reference moving with a velocity $\mathbf{v}$. Denoting the partial molar volume of the kth constituent as $\bar{v}_k$ it can be noted that

$$\mathbf{v}=\sum_k \bar{v}_k \mathbf{J}'_k$$

and (68)

$$0=\sum_k \bar{v}_k \mathbf{J}_k$$

Relating $\mathbf{J}'_k$ and $\mathbf{J}_k$ is the expression

$$\mathbf{J}'_k = C_k \mathbf{v} + \mathbf{J}_k \tag{69}$$

The quantity $\mathbf{v}$ is obtained from the appropriate equations of classical mechanics and hydrodynamics or by direct measurement.

If the flux of every constituent relative to a fixed reference frame can be represented as shown in Eq. (69) by a value $\mathbf{v}$ that is spatially uniform for every element of volume of the system, it is clear that this amounts only to giving the entire system a translational velocity involving no movement of the constituents relative to one another other than that produced by the fluxes $\mathbf{J}_k$. Limitations of space do not permit a general discussion of the relation between convective and diffusional components of flux; however, the particular case of osmotic solvent transfer is of sufficient biological interest to deserve a brief comment.

If $\mu_k(P)$ is the chemical potential of the kth constituent at some pressure P and $\mu_k(P_0)$ is its chemical potential at the standard pressure P_0 then

$$\mu_k(P)=\mu_k(P_0)+\int_{P_0}^{P} \frac{\partial \mu}{\partial P}_{T\ n_j \neq k}\, dP \tag{70}$$

From the definition in Eq. (10) of μ_k, the relations in Eq. (8), and the fact that the order of differentiation can be interchanged without changing the value of the derivative, one obtains that

$$\left(\frac{\partial \mu_k}{\partial P}\right)_{T,\ n_j\neq k} = \left(\frac{\partial V}{\partial n}\right)_{T,\ P,\ n_j\neq k} = \bar{v}_k \tag{71}$$

Assuming $\bar{v}_k$ to be constant over the range of pressures from P_0 to P, Eq. (70) becomes

$$\mu_k(P)=\mu_k(P_0)+(P-P_0)\bar{v}_k \tag{72}$$

Denoting the mole fraction of the kth constituent as χ_k, the chemical potential can be written (cf. MacDougall, 1939, p. 144) in the form

$$\mu_k(P_0) = \mu_k^0 + RT \ln \chi_k \tag{73}$$

which assumes the solution to be ideal, but the kth constituent need not be dilute.

Consider a membrane, permeable only to the kth constituent identified here with the solvent, with a pressure difference $\Delta P = P_1 - P_2$ between the two sides 1 and 2. If $(\chi_k)_1$ and $(\chi_k)_2$ are the mole fractions of the kth constituent on the two sides, the difference $\Delta\mu_k$ in the chemical potential across the membrane can, from Eqs. (72) and (73), be written as

$$\Delta\mu_k = RT \ln \frac{(\chi_k)_1}{(\chi_k)_2} + \bar{v}_k \Delta P$$

Regarding $\Delta\mu_k$ as the force " driving " the transfer of solvent across the membrane and assuming the solvent flux proportional to $\Delta\mu_k$ one obtains

$$J_k = L_k \Delta\mu_k$$

without questioning the mechanism by which the flow occurs. Recent experiments (Mauro, 1957) indicate that water transfer through a collodion membrane under such circumstances is not accomplished via diffusion in the strict sense of the word but by a process more nearly resembling a convective flow. Mauro refers to such a flow as " semilaminar flow ". It should be emphasized, however, that the question of whether the flux in this particular case is primarily diffusional or " semilaminar " affects only the interpretation of the coefficient L_k since the expression for each of the two types of flow is proportional to $\Delta\mu_k$.

VI. Modern Addenda to Classical Thermodynamics

A. Introductory Comments

In recent years several noteworthy additions have been made to the axiomatic foundations of thermodynamics with important implications for application to biological systems.

It will be recalled that the assertion of the second law of thermodynamics by means of Eq. (2) is an inequality. By introducing a quantity called the " uncompensated heat " deDonder (deDonder and Van Rysselberghe, 1936) has introduced an equality statement of the second law. Closely associated with the uncompensated heat is a thermodynamic quantity called the " irreversible entropy ". In the hands of writers such as deDonder and Van Rysselberghe (1936), Tolman and Fine (1948), and Prigogine (1955) this equality statement has evolved into a formulation that places a much greater restriction upon the processes possible in nature than does the second law.

It will be instructive to examine some of the properties of this reformulation of the second law. In this exposition the writings of deDonder and Van Rysselberghe (1936), Prigogine (1955), and Denbigh (1951) will be leaned upon heavily.

B. The Equality Statement of the Second Law

To begin with it will be convenient to give a statement of the first law that is somewhat more appropriate for open systems. The assertion of the first law given by Eq. (1) assumes that heat and work are the only avenues of exchange of energy between the system and the environment and is thus applicable only to closed systems. Let us by the symbol $đ\Phi$ denote the contribution to the energy of the system from both heat transfer and exchange of matter across the boundaries of the system from the environment. By replacing $đQ$ in Eq. (1) with $đ\Phi$ one obtains

$$dE = đ\Phi - đW \tag{74}$$

as the proper statement of the first law for an *open* system.

One can regard the entropy change of a system as the sum of two components. The differential change in the entropy of the system arising from a reversible transfer of heat or matter across the boundary from the environment shall be denoted as d_eS. The differential change in entropy due to processes internal to the system is called the irreversible entropy change and denoted as d_iS. Thus

$$dS = d_eS + d_iS \tag{75}$$

The relation between the uncompensated heat, $đQ^*$ of deDonder and Van Rysselberghe (1936) and the irreversible entropy of Tolman and Fine (1948) is that

$$d_iS = \frac{đQ^*}{T} \tag{76}$$

It is stated as a fundamental postulate that

$$d_iS \geqslant 0 \tag{77}$$

for any real process in any kind (open, closed, or isolated) of system capable of thermodynamic description. This is indeed a bold assertion of great generality.

In the case that a system is isolated there will be no exchanges of heat, work, or matter with the surroundings, so that $d_eS=0$ and $d_eS=d_iS\geqslant 0$.

Consider a system, isolated from the environment, divided into two subsystems I and II such that systems I and II are free to exchange matter and energy with each other. Under these circumstances

$$dS=dS_{\mathrm{I}}+dS_{\mathrm{II}} \tag{78}$$

$$d_eS_{\mathrm{I}}=-d_eS_{\mathrm{II}} \tag{79}$$

Hence from Eqs. (75), (78), and (79) one obtains that

$$dS=d_iS_{\mathrm{I}}+d_iS_{\mathrm{II}} \tag{80}$$

We now note an interesting difference between the restrictions imposed by Eqs. (77) and (2). Equation (2), i.e., the classical statement of the second law, requires only that

$$dS=d_iS_{\mathrm{I}}+d_iS_{\mathrm{II}}>0 \tag{81}$$

It offers no prohibitions against situations of the sort in which one might have $d_iS_{\mathrm{I}}<0$ and $d_iS_{\mathrm{II}}>0$ provided only that their sum is positive. On the other hand, such situations are prohibited by Eq. (77) which requires that

$$d_iS_{\mathrm{I}}>0 \quad \text{and} \quad d_iS_{\mathrm{II}}>0 \tag{82}$$

Thus one can look upon the classical second law as a " global " formation which imposes an over-all restriction on the behavior of the system as a whole and Eq. (77) as a " local " formulation that places a restriction on the behavior of every element of volume of the system. Any process which is in accord with Eq. (77) will also be in agreement with the classical second law, but the converse is not necessarily true.

Let us now consider an open system, capable of doing work, in which a chemical reaction can occur. Although only a single reaction will be considered in the present case, the extension to any number of reactions, along the lines discussed previously, is straightforward and does not affect the generality of the results.

Differentiating Eqs. (4) and (5), setting dP and dT equal to 0, and solving for TdS one obtains

$$TdS=dE+PdV-(dF)_{T,P} \tag{83}$$

Equation (15) may be substituted for $(dF)_{T,P}$ so

$$dS\geqslant\frac{1}{T}(dE+PdV+\text{đ}W')-\frac{1}{T}\sum\mu_j dn_j \tag{84}$$

Using the defining relation in Eq. (6) and Eq. (74), Eq. (84) can be written in the form

$$dS \geqslant \frac{1}{T} đ\Phi - \frac{1}{T} \sum \mu_j dn_j \tag{85}$$

Noting that (for demonstration see any of the standard textbooks such as MacDougall, 1939; Guggenheim, 1950; or Fermi, 1937)

$$\left(\frac{\partial S}{\partial n_j}\right)_{E,V,n_k} = -\frac{1}{T}\left(\frac{\partial F}{\partial n_j}\right)_{P,T,n_k} = -\mu_j/T$$

Eq. (85) for open systems can be written in the form

$$dS \geqslant \frac{1}{T} đ\Phi - \sum_j \left(\frac{\partial S}{\partial n_j}\right)_{E,V,n_{k \neq j}} dn_j$$

which should be compared with Eq. (2) asserted for closed systems. The change in the number of moles of any constituent will be the amount of that constituent added to the system from the environment plus the amount produced by the chemical reaction. Thus one can write that

$$dn_j = d_e n_j + m_j d\xi \tag{86}$$

For entropy imparted to the system reversibly from the environment one can write

$$d_e S = \frac{1}{T} đ\Phi - \frac{1}{T} \sum_j \mu_j d_e n_j \tag{87}$$

Hence, from Eqs. (85), (86), and (87) one obtains that the irreversible entropy change is

$$d_i S \geqslant -\frac{1}{T} \sum_j \mu_j m_j d\xi \tag{88}$$

If the chemical reaction is the only irreversible process occurring in the system then

$$d_i S = -\frac{1}{T} \sum_j \mu_j m_j d\xi \tag{89}$$

and since $d_i S \geqslant 0$ it must be true that

$$\sum_j \mu_j m_j d\xi \leqslant 0 \tag{90}$$

which, as we know, is also required by the classical statement of the second law.

It is worth noting the connection between the rate of creation of irreversible entropy and the rate of dissipation of free energy in a chemical reaction system at constant temperature and pressure. This is

$$\frac{d_iS}{dt} = -\frac{1}{T}\frac{dF}{dt} \tag{91}$$

as may be seen from Eqs. (89) and (31) or from the discussion of Denbigh (1951, p. 46).

C. Microscopic Reversibility and Detailed Balancing

Another principle not a part of the classical framework of thermodynamics is known as "the principle of microscopic reversibility" (Tolman, 1924, 1938), one that is very closely related to another called "the principle of detailed balancing" (Fowler, 1924, 1929; Lewis, 1925). Although these notions, having their origin in the theory of statistical mechanics, are less general than the first two laws of thermodynamics, they are nevertheless applicable under a wide variety of circumstances and will deserve a brief discussion.

In the course of this discussion the word "state" will be used in a somewhat different sense than it has been used so far in this chapter. All of the discussion up to this point has been concerned with macroscopic systems and macroscopic variables. The discussion in this section shall be primarily concerned with microscopic systems and refer to the state of a molecule. The state of a molecule is specified if its position, internal configuration, and relevant velocity components are known. In terms of quantum mechanics and speaking with a rigor unnecessary for the present discussion one can say that a molecule is in a completely specified state when all of its relevant quantum numbers have been specified (Mayer and Mayer, 1940, p. 44). By contrast to the macroscopic system that can be considered to undergo a more or less gradual transition between states, the state transition of a molecule can be viewed as a more or less abrupt event. Equilibrium in a macroscopic system means a zero rate of change, while, if one speaks of equilibrium between two microscopic states, one means that the average number of transitions per unit time resulting in passage from state 1 to state 2 is the same as that from 2 to 1. A homogeneous macroscopic system of a single chemical constituent will, when the number of moles, the volume, and the temperature are specified, be in a defined thermodynamic state. The molecules of the system will, however, be in a number of different states. The proportion of molecules having a particular energy value will, for example, be given by the Maxwell-Boltzman (e.g., cf. Mayer and Mayer, 1940) distribution.

According to Tolman (1938), two states of a molecule are called *congruent* if they can be made identical by a translation and rotation in space, *reverse* if the molecule can be changed from one of the states to a state congruent with the other by only a reversal in direction of all components of velocity. Let $Z_{i,\,k}$ denote the number of direct transitions from state i to state k. Let $-i$ and $-k$ respectively denote the reverse states of i and k. The *principle of microscopic reversibility* states that

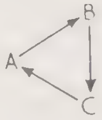
$$Z_{i,\,k}=Z_{-k,\,-i}$$

at equilibrium. This relation can be used to demonstrate (cf. Tolman, 1938) the *principle of detailed balancing*, i.e., that

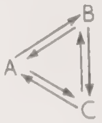
$$Z_{\pm i,\,\pm k}=Z_{\pm k,\,\pm i}$$

Since the energy and chemical configuration will be the same for a molecular state and its reverse, we shall not be interested in distinguishing between two such states. The principle of detailed balancing can therefore be stated as follows. *At equilibrium, the average number of direct transitions per unit time from molecular condition A to molecular condition B will be equal to the number of such transitions from B to A. The transitions from A to B do not have to be thought of as balanced with the help of some indirect route such as B to C to A.*

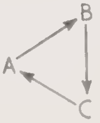
An example discussed by Denbigh (1951, pp. 31–38), serves to illustrate this principle very nicely. Consider a substance capable of existence in the three tautomeric forms A, B, and C in a single homogeneous, phase. Suppose interconversion can occur between these three states of the substance and the relative concentrations of each have adjusted so that the three forms are in equilibrium with one another. The conditions for thermodynamic equilibrium would be satisfied if, at the molecular level, the rates of each of the transitions around the cycle were



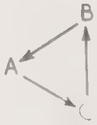
equal. The principle of detailed balancing, however, requires that the forward and reverse rate of each step be the same. Thus equilibrium must be represented as



rather than



or



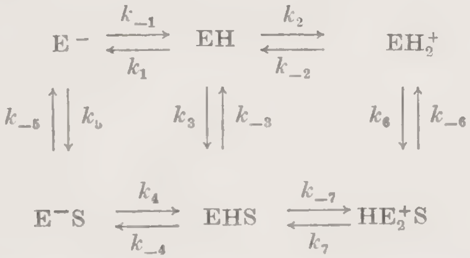
This condition imposes a restriction on reaction systems that is of considerable importance in metabolism where the conceivable alternative pathways linking two molecular states may be fairly numerous. Denote the rate constants for the clockwise transitions as k_1, k_2, and k_3 and for the respective reverse steps as k_{-1}, k_{-2}, and k_{-3}. The three equilibrium constants K_1, K_2, and K_3 can be written

$$K_1 = k_{-1}/k_1, \quad K_2 = k_{-2}/k_2, \quad \text{and} \quad K_3 = k_{-3}/k_3$$

as soon as the necessity for detailed balancing is agreed upon. For the second law to be consistent with the results of detailed balancing, it must also be true that

$$k_1 k_2 k_3 = k_{-1} k_{-2} k_{-3} \tag{92}$$

Another example may serve to clarify the nature of the restriction placed on metabolic schemes by this principle. Most enzymes have some pH value for which their rate of catalysis is a maximum. Perhaps the simplest reaction scheme for the interpretation of such a phenomenon is to suppose that hydrogen ions can dissociate from the enzyme so it may be electrically negative, neutral, or positive, depending upon the pH. The reactions in which the hydrogen ion dissociates and the enzyme substrate complex is formed might be represented by the reaction scheme depicted as



with the rate constants $k_1, k_{-1}, \ldots, k_{-7}$ adjacent to the arrows indicating the process to which they refer. The equilibrium constants of the various reactions will be $K_1 = k_1/k_{-1}$, $K_2 = k_2/k_{-2}$, etc. in accord with the requirement of detailed balancing. In order that the second law of thermodynamics be satisfied it is necessary that

$$dF_3/d\xi_3 = dF_1/d\xi_1 + dF_5/d\xi_5 + dF_4/d\xi_4$$
$$dF_3/d\xi_3 = dF_2/d\xi_2 + dF_6/d\xi_6 + dF_7/d\xi_7$$

in which

$$dF_1/d\xi_1 = -RT \ln K_1 + RT \ln C_{\mathrm{E}} C_{\mathrm{H}}/C_{\mathrm{EH}},$$
$$dF_3/d\xi_3 = -RT \ln K_3 + RT \ln C_{\mathrm{EHS}}/C_{\mathrm{EH}} C_{\mathrm{S}}, \text{ etc.}$$

This condition is, however, completely equivalent to the requirement that

$$K_3 = K_1K_4K_5 \quad \text{and} \quad K_3 = K_2K_6K_7 \tag{93}$$

or $$k_3/k_{-3} = k_1k_4k_5/k_{-1}k_{-4}k_{-5} \quad \text{and} \quad k_3/k_{-3} = k_2k_6k_7/k_{-2}k_{-6}k_{-7}$$

Thus, instead of 14 independent rate constants there are only 12, the other two being determined by these two necessary relations between the equilibrium constants. Since, in complicated metabolic reaction schemes, such closed reaction loops may occur fairly frequently, there will be a considerable number of restrictive relations of the form of Eqs. (92) and (93) to delimit the domain of values which, consistent with physical reality, the rate constants may have. As will be seen in the discussion of the section to follow, the principle of microscopic reversibility also places important restrictions upon processes other than chemical reactions.

D. Generalized Transfer Equations

Although it is frequently assumed for experimental investigations that processes such as diffusion, heat conduction, osmosis, electrical conduction, occur independently of one another, there are a number of phenomena in physical and biological systems that depend upon interaction or coupling between these different processes. Thus, although the Fourier equation

$$\mathbf{J} = -\sigma \nabla T$$

is commonly used to measure the thermal conductance of a material, it is well known that, when two dissimilar metals are placed in contact and heated, the flow of heat becomes coupled with a flow of electric charge in what is known as the *thermoelectric effect*. Similarly, although Fick's law [see Eq. (53)] is used to describe and to investigate experimentally the diffusion of a material, the diffusion may also be coupled with heat conduction in the phenomenon called the Soret effect or *thermoosmosis*. In still other cases one may have coupling between the diffusion of several different chemical species, etc.

In a number of instances in living systems it is known that coupling of fluxes occurs, but, for the most part, the mechanism by which this coupling is accomplished is poorly understood. The flux of glucose across the wall of the intestine, the exchange of ions and transport of water across the wall of the kidney tubule and of charge through the frog skin are all familiar examples of coupled transport processes in biological systems. In most biological cases it is not known just what processes are coupled to those of the type mentioned, but because the observed flow is frequently from regions of low to high chemical potential, it can be inferred that coupling with one or more exergonic processes must occur. The possible role of coupled diffusion processes in the accumulation of materials against their concentration gradients in the cell has already been pointed out by Hearon (1950b).

It has been suggested previously in the chapter that many processes involving the transport of quantities such as cellular constituents can be written in the form

$$\mathbf{J} = \Omega C \mathbf{X} \tag{94}$$

where C is the concentration, $\mathbf{J}$ is the flux, $1/\Omega$ is regarded as a " frictional coefficient " and $\mathbf{X}$ as the " driving force ". One might write Eq. (94) in the simplified form

$$\mathbf{J} = L\mathbf{X} \tag{95}$$

where $L = \Omega C$ when $\mathbf{J}$ refers to the flux of some chemical constituent. If $\mathbf{J}$ refers to a flux of charge, i.e., current intensity, then L would be identified with the specific conductance of the medium, etc.

Consider two irreversible processes involving the transport of two quantities whose fluxes and driving forces shall be denoted as $\mathbf{J}_1$, $\mathbf{J}_2$, $\mathbf{X}_1$, and $\mathbf{X}_2$ respectively. It has been suggested (cf. Onsager and Fuoss, 1932 ; Denbigh, 1951) as a generalization of Eq. (95) that the phenomenological equations

$$\begin{aligned} \mathbf{J}_1 &= L_{11}\mathbf{X}_1 + L_{12}\mathbf{X}_2 \\ \mathbf{J}_2 &= L_{21}\mathbf{X}_1 + L_{22}\mathbf{X}_2 \end{aligned} \tag{96}$$

be used to describe such a pair of processes. If the two processes are independent, i.e., if no coupling occurs between them, then

$$L_{12} = 0 \quad \text{and} \quad L_{21} = 0 \tag{97}$$

and the system of Eqs. (96) reduces to

$$\begin{aligned} \mathbf{J}_1 &= L_{11}\mathbf{X}_1 \\ \mathbf{J}_2 &= L_{22}\mathbf{X}_2 \end{aligned} \tag{98}$$

which are of the same form as Eq. (95).

As a generalization of Fick's law [see Eq. (53)] in the case of the diffusion of two constituents, Onsager has suggested the system of equations

$$\begin{aligned} \mathbf{J}_1 &= -D_{11}\nabla C_1 - D_{12}\nabla C_2 \\ \mathbf{J}_2 &= -D_{21}\nabla C_1 - D_{22}\nabla C_2 \end{aligned} \tag{99}$$

where D_{11} and D_{22} will be the conventional diffusion coefficient of the first and second constituent, respectively, under the circumstances that D_{12} and D_{21} are zero. If D_{12} and D_{21} are not zero, then coupling will occur between the two diffusion flows.

Thus in these generalizations of the flux equations one has a square array or matrix of coefficients that relates the fluxes of the various processes to the driving forces. Under the circumstances that no coupling occurs, the " off diagonal " terms, i.e., L_{12} and L_{21} or D_{12} and D_{21}, will be zero. Onsager (1931a, b) has demonstrated that the principle of microscopic reversibility mentioned earlier implies that the matrix of coefficients of a system of flux equations like Eq. (96) must be symmetric. In the particular case of Eq. (96) this means that $L_{21} = L_{12}$. More will be said about this later after presentation of some examples of coupled processes.

The following example is based on a discussion by Prigogine (1955, p. 63). Consider a membrane or plate made of some porous or gel-like substance containing ionizable chemical groups. A membrane containing an appreciable amount of protein or made of an ion exchange resin would present such a situation. The dissociation of ionizable groups results in the membrane having a net charge and the water phase an equal and opposite charge. If electric current is passed through such a membrane, streaming of water across the membrane will occur. This effect is of considerable interest in the experimental investigation of the electrostatic potential, the so-called " zeta potential ," in the micro domain of the surface of the colloid comprising the gel. For a discussion in this regard see Glasstone (1946). The experiments of Tobias and Solomon (1950) on optical and mechanical changes in nerve suggest that such streaming effects may also occur in nerve excitation.

In such a situation there are two irreversible processes, transport of charge and transport of matter, coupled together and under the influence of a difference in hydrostatic pressure and a difference in electric potential. Designate

I = electric current per unit area of membrane

J = fluid flow per unit area of membrane (measured in volume per unit area per unit time)

$\Delta\Psi$ = difference in electric potential across the membrane

ΔP = difference in pressure across the membrane

As phenomenological equations one can write

$$\begin{aligned} I &= L_{11}\frac{\Delta\Psi}{T} + L_{12}\frac{\Delta P}{T} \\ J &= L_{21}\frac{\Delta\Psi}{T} + L_{22}\frac{\Delta P}{T} \end{aligned} \tag{100}$$

with the Onsager relation $L_{12} = L_{21}$. One might apply some pressure difference and then measure the electrical potential difference developed when no electric current is allowed to flow. The increase in potential for a given increase in pressure under these circumstances is called the *streaming potential* and is given by the relation

$$\left[\frac{\Delta\Psi}{\Delta P}\right]_{I=0} = \frac{L_{12}}{L_{11}} \tag{101}$$

On the other hand one might have no pressure difference and ask the increase in the fluid flow as the current is increased. The quantitative measure of this effect, called *electroosmosis*, is given by the relation

$$(J/I)_{\Delta P=0} = L_{21}/L_{11} \tag{102}$$

If the flow is zero and a potential difference is applied then a pressure difference will be developed. This pressure is called the *electroosmotic pressure* and, in terms of the Onsager coefficients, is given by the relation

$$\left[\frac{\Delta P}{\Delta\Psi}\right]_{J=0} = -\frac{L_{21}}{L_{22}} \tag{103}$$

If a pressure difference is applied, but the two sides "shorted out", so that $\Delta\Psi = 0$, then there will occur both a flow of matter and electric current. This current is called the *streaming current* and is given by the relation

$$\left[\frac{I}{J}\right]_{\Delta\Psi=0} = \frac{L_{12}}{L_{22}} \tag{104}$$

From the Onsager requirements that $L_{12} = L_{21}$ and Eqs. (101), (102), (103), and (104) we can also note that

$$\left[\frac{\Delta\Psi}{\Delta P}\right]_{I=0} = -\left[\frac{J}{I}\right]_{\Delta P=0} \tag{105}$$

and

$$\left[\frac{\Delta P}{\Delta\Psi}\right]_{J=0} = -\left[\frac{I}{J}\right]_{\Delta\Psi=0} \tag{106}$$

It will be instructive to examine the Onsager generalizations of Fick's law in Eq. (99) and two methods by which such diffusion coupling can occur. The simplest and most obvious mechanism by which coupling between two diffusion species might occur is that of complex formation between the species. It will therefore be of interest to derive expressions of the form of Eqs. (96) and (99) for such a situation.

Consider a system containing two species of solute in solution and suppose that a third species can be formed by the complexing of the two original species. Designate

$\mathbf{J}_1^*$ = flux of uncombined solute species 1
$\mathbf{J}_2^*$ = flux of uncombined solute species 2
$\mathbf{J}_{12}$ = flux of complex of species 1 and 2
$\mathbf{J}_1$ = total flux of solute species 1, including both free and complexed
$\mathbf{J}_2$ = total flux of solute species 2, including both free and complexed
Ω_1 = mobility of uncombined species 1
Ω_2 = mobility of uncombined species 2
Ω_{12} = mobility of complex
C_1 = concentration of uncombined species 1
C_2 = concentration of uncombined species 2
C_{12} = concentration of complex
μ_1 = chemical potential of uncombined species 1
μ_2 = chemical potential of uncombined species 2
μ_{12} = chemical potential of complex
K = equilibrium constant for dissociation of the complex

For the example at hand we shall suppose that one molecule of the first species complexes with one molecule of the second species. It will be assumed also that the complex is effectively in equilibrium with the dissociated forms in every region of the system. One can thus write that

$$\begin{aligned} \mathbf{J}_1 &= \mathbf{J}_1^* + \mathbf{J}_{12} \\ \mathbf{J}_2 &= \mathbf{J}_2^* + \mathbf{J}_{12} \end{aligned} \tag{107}$$

and from the local equilibrium assumption

$$\mu_{12} = \mu_1 + \mu_2 \tag{108}$$

$$\frac{C_1 C_2}{C_{12}} = K \tag{109}$$

If the formation of the complex is the only way that coupling between species 1 and 2 can occur then one can write

$$\mathbf{J}_1^* = -\Omega_1 C_1 \nabla\mu_1 \tag{110}$$

$$\mathbf{J}_2^* = -\Omega_2 C_2 \nabla\mu_2 \tag{111}$$

$$\mathbf{J}_{12} = -\Omega_{12} C_{12} \nabla\mu_{12} \tag{112}$$

Since $\nabla\mu_{12} = \nabla\mu_1 + \nabla\mu_2$ and C_{12} can be expressed in terms of C_1 and C_2 by use of Eq. (109) one can, by substitution of Eqs. (112), (110), and (111) into (107), obtain that

$$\begin{aligned} \mathbf{J}_1 &= -\left(\Omega_1 C_1 + \frac{\Omega_{12}}{K} C_1 C_2\right)\nabla\mu_1 - \frac{\Omega_{12}}{K} C_1 C_2 \nabla\mu_2 \\ \mathbf{J}_2 &= -\left(\Omega_2 C_2 + \frac{\Omega_{12}}{K} C_1 C_2\right)\nabla\mu_2 - \frac{\Omega_{12}}{K} C_1 C_2 \nabla\mu_1 \end{aligned} \tag{113}$$

By comparison of Eqs. (113) with (96) it can be seen that for the case at hand

$$\begin{aligned} L_{11} &= \left(\Omega_1 C_1 + \frac{\Omega_{12}}{K} C_1 C_2\right) \\ L_{12} = L_{21} &= \frac{\Omega_{12}}{K} C_1 C_2 \\ L_{22} &= \left(\Omega_2 C_2 + \frac{\Omega_{12}}{K} C_1 C_2\right) \end{aligned} \tag{114}$$

If the two unbound solutes behave as if in perfect solution, i.e., their chemical potentials can be written in the form of Eq. (20), and their standard chemical potentials are spatially uniform through the system, then the flux equations (113) can be written in the form

$$\begin{aligned} \mathbf{J}_1 &= -RT\left(\Omega_1 + \frac{\Omega_{12} C_2}{K}\right)\nabla C_1 - RT\,\frac{\Omega_{12} C_1}{K} \nabla C_2 \\ \mathbf{J}_2 &= -RT\frac{\Omega_{12} C_2}{K} \nabla C_1 - RT\left(\Omega_2 + \frac{\Omega_{12} C_1}{K}\right)\nabla C_2 \end{aligned} \tag{115}$$

Comparison of the system in Eqs. (115) with the Onsager generalization in Eqs. (99) of Fick's law shows that in this case

$$
\begin{aligned}
D_{11} &= RT\left(\Omega_1 + \frac{\Omega_{12}C_2}{K}\right) \\
D_{12} &= RT\,\frac{\Omega_{12}C_1}{K} \\
D_{21} &= RT\,\frac{\Omega_{12}C_2}{K} \\
D_{22} &= RT\left(\Omega_2 + \frac{\Omega_{12}C_1}{K}\right)
\end{aligned}
\tag{116}
$$

Another manner in which coupling between two diffusing constituents may occur is by a mutual effect on the activity coefficients of one another. Such effects can be expected to be relatively prominent in the case of electrolytes where, even in relatively dilute solution, all the charged species contribute to the activity coefficients of one another in the manner considered by Debye and Hückel (cf. MacDougall, 1939). Limitations of space do not permit detailed treatment of such problems here and the interested reader is referred to the discussions of Onsager and Fuoss (1932) and Lamm (1947).

Suppose one considers two constituents 1 and 2 such that the chemical potentials of each are of the form

$$
\begin{aligned}
\mu_1 &= \mu_1^0 + RT \ln \gamma_1 C_1 \\
\mu_2 &= \mu_2^0 + RT \ln \gamma_2 C_2
\end{aligned}
\tag{117}
$$

and the activity coefficients γ_1 and γ_2 are different from unity and are functions of the concentrations C_1 and C_2. Assuming $\nabla\mu_1^0 = \nabla\mu_2^0 = 0$ one can write that

$$
\begin{aligned}
\nabla\mu_1 &= \frac{\partial\mu_1}{\partial C_1}\nabla C_1 + \frac{\partial\mu_1}{\partial C_2}\nabla C_2 \\
\nabla\mu_2 &= \frac{\partial\mu_2}{\partial C_1}\nabla C_1 + \frac{\partial\mu_2}{\partial C_2}\nabla C_2
\end{aligned}
\tag{118}
$$

in which, by differentiation of the expressions in Eqs. (117)

$$
\begin{aligned}
\frac{\partial\mu_1}{\partial C_1} &= RT\left(\frac{1}{C_1} + \frac{\ln \gamma_1}{C_1}\right) \\
\frac{\partial\mu_1}{\partial C_2} &= RT\,\frac{\partial \ln \gamma_1}{\partial C_2}, \text{ etc.}
\end{aligned}
\tag{119}
$$

Substituting Eqs. (119) and (118) into the two flux equations of the type of Eqs. (96) for the two constituents one finds that

$$\begin{aligned} \mathbf{J}_1 &= -D_1\left(1+C_1\frac{\partial \ln \gamma_1}{\partial C_1}\right)\nabla C_1 - D_1C_1\frac{\partial \ln \gamma_1}{\partial C_2}\nabla C_2 \\ \mathbf{J}_2 &= -D_2C_2\frac{\partial \ln \gamma_2}{\partial C_1}\nabla C_1 - D_2\left(1+C_2\frac{\partial \ln \gamma_2}{\partial C_2}\right)\nabla C_2 \end{aligned} \tag{120}$$

A comparison with Eqs. (99) shows the Onsager diffusion coefficients to be

$$\begin{aligned} D_{11} &= D_1\left(1+C_1\frac{\partial \ln \gamma_1}{\partial C_1}\right) \\ D_{12} &= D_1C_1\frac{\partial \ln \gamma_1}{\partial C_2}, \text{ etc.} \end{aligned} \tag{121}$$

Since

$$\left[\frac{\partial \mu_j}{\partial n_k}\right]_{T,P} = \frac{\partial}{\partial n_k}\left[\frac{\partial F}{\partial n_j}\right] = \frac{\partial}{\partial n_j}\left[\frac{\partial F}{\partial n_k}\right] = \left[\frac{\partial \mu_k}{\partial n_j}\right]_{T,P}$$

it should be noted in Eqs. (121) that

$$\frac{\partial \ln \gamma_1}{\partial C_2} = \frac{\partial \ln \gamma_2}{\partial C_1}$$

With the previous discussion concerning the contingency of diffusion coupling upon the coefficients D_{12} and D_{21} it can be seen from Eqs. (121) that if γ_1 is dependent upon C_2 and/or γ_2 is dependent upon C_1, then diffusion coupling will occur between the two species.

Although, for simplicity, only systems of two coupled fluxes have been considered, it should be noted that the extension of the theoretical notions to an arbitrary number of such fluxes is straightforward. Thus if the flux and driving force of the kth constituent are denoted as $\mathbf{J}_k$ and $\mathbf{X}_k$ repectively one could consider the generalized system

$$\mathbf{J}_k = \sum_{j=1}^{n} L_{kj}\mathbf{X}_j \qquad k = 1, \ldots, n \tag{122}$$

The Onsager symmetry requirement for the system would be $L_{kj} = L_{jk}$.

E. The Principle of Minimum Entropy Production

Prigogine (1955) has suggested a variational formulation for irreversible processes that is interesting although of limited utility. Because of these limitations it will be outlined only briefly in the present exposition.

Statement 1. Consider a system of fluxes such as that in Eq. (122). Suppose that some subset, e.g., $\mathbf{X}_1, \dots, \mathbf{X}_s$, of the set of forces $\mathbf{X}_1, \dots \mathbf{X}_n$ is held constant and the other $\mathbf{X}_{s+1}, \dots, \mathbf{X}_n$ allowed to take on whatever values they will as the system adjusts to its " steady state ". Prigogine asserts that *the values which* $\mathbf{X}_{s+1}, \dots, \mathbf{X}_n$ *will take on in the steady state, within the constraints imposed by the system, are such that the rate of irreversible entropy production is a minimum, the " steady state " being that state in which* $\mathbf{J}_{s+1} = \dots = \mathbf{J}_n = 0$.

Statement 2. Consider a system of reactions with rates $u_1, \dots, u_n$ and affinities $\alpha_1, \dots, \alpha_n$ such that

$$u_j = \sum_{i=1}^{n} L_{ij}\alpha_i \qquad j = 1, \dots, n \tag{123}$$

As previously (see Eq. 29) let m_{jk} denote the stoichiometric coefficient of the kth substance in the jth reaction. One can then write that

$$-\alpha_j = \sum_{k=1}^{M} m_{jk}\mu_k \qquad j = 1, \dots, n \tag{124}$$

and

$$\frac{dC_k}{dt} = \sum_{j=1}^{n} m_{jk}u_j \qquad k = 1, \dots, M \tag{125}$$

The rate of production of irreversible entropy will then be

$$\frac{d_iS}{dt} = \frac{1}{T}\sum_{j} \alpha_j u_j \tag{126}$$

Suppose that the affinities or the chemical potentials are restricted by assigning fixed values to some of them or requiring that certain linear constraints, e.g., $\sum_j \alpha_j =$ constant, be satisfied. Within these constraints the chemical potentials (and consequently the affinities) of the system are allowed to take on whatever value they will. Under these circumstances the Prigogine formulation asserts that *the values which the affinities will take on in the steady state are such that the rate of irreversible* *entropy production is a minimum.* It can be demonstrated for a number of circumstances that, provided the phenomenological relations in Eq. (123) are appropriate, this definition of the steady state is equivalent to the conventional requirement that $dC_k/dt = 0$ for all k.

Several remarks should be appended to these two statements. In the first place it should be pointed out that Prigogine's fundamental definition of the steady state in Statement 1 is different from that of the present authors who prefer the conventional usage already existent in chemical kinetics and biochemistry, i.e., that the distributions of the relevant quantities in the system be time-independent. Thus, if the fluxes refer to various chemical constituents one would require that the time derivative, $\partial C_k/\partial t$, of the concentration C_k be zero at every point in the system for every k. For flows of heat or electric charge one would require that the time derivatives of the temperature and space charge respectively be zero. In view of Eqs. (60), (61), and (65) this means a stipulation with respect to the divergence of the fluxes rather than Prigogine's requirement upon the fluxes themselves. Under some circumstances both definitions of the steady state give the same result, in others they do not.

Another and perhaps more severe criticism of the minimum entropy production rate principle is that it depends for its validity upon phenomenological equations for the various rates that are linear in the driving forces. This assumption probably introduces no difficulty in Statement 1, but in Statement 2 this means that the rate must be linear in the affinities of the reactions and that the coefficients L_{ij} of Eq. (123) must be constants. Since one knows that reaction rates are primarily proportional to the concentrations, the requirements of Eq. (123) can be satisfied only when the reaction is in the neighborhood of equilibrium. It is possible, since the affinities of the individual steps in metabolic processes are frequently small, that this requirement may be approximately satisfied, but one should be cautious in assuming that it is.

As an illustrative example of the way in which this principle is used we may consider the following situation discussed by Prigogine (1955).

Consider the sequence of reactions in which

$$
\begin{array}{l}
R_1 \rightleftarrows R_2 \\
R_2 \rightleftarrows R_3 \\
\vdots \\
R_n \rightleftarrows R_{n+1}
\end{array}
$$

Denote the affinities and the rates of the various steps as $\alpha_1, \alpha_2, \ldots, \alpha_n$ and $u_1, u_2, \ldots, u_n$ respectively. Suppose that R_1 is supplied to the system and R_{n+1} is removed so that the over-all affinity

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$$\alpha = \sum_{j=1}^{n} \alpha_j$$

is held constant at some preassigned value. Suppose also that the individual steps are sufficiently close to equilibrium that their rates can be assumed to be proportional to their affinities, i.e., that

$$u_j = L_j \alpha_j, \qquad j = 1, \ldots, n \tag{127}$$

The rate of irreversible entropy production in the system will be

$$\frac{d_i S}{dt} = \frac{1}{T} \sum u_j \alpha_j = \frac{1}{T} \sum L_j \alpha_j^2 \tag{128}$$

Consider now the change in the system arising from a set of infinitesimal variations $d\alpha_1, \ldots, d\alpha_n$ in the affinities $\alpha_1, \ldots, \alpha_n$. When the rate of irreversible entropy production is at its minimum value then the change in it produced by the set of changes $d\alpha_1, \ldots, d\alpha_n$ must be zero. Thus must

$$d\left[\frac{d_i S}{dt}\right] = \frac{1}{T} \sum_j \frac{\partial}{\partial \alpha_j} \left[\frac{d_i S}{dt}\right] d\alpha_j = 0 \tag{129}$$

for any set of variations of the affinities consistent with the requirement that

$$d\alpha = \sum d\alpha_j = 0 \tag{130}$$

From Eqs. (128) and (129) it is clear that the condition in Eq. (129) is completely equivalent to the requirement that

$$\sum L_j \alpha_j d\alpha_j = 0 \tag{131}$$

for any arbitrary set of variations $d\alpha_1, \ldots, d\alpha_n$ consistent with Eq. (130). If it were not for the condition in Eq. (130) then Eq. (131) could be true for *every* set $d\alpha_1, \ldots, d\alpha_n$ if, and only if, $L_j \alpha_j = 0$ for every j.[†] The constraint of Eq. (130) can be introduced into Eq. (131) by the ingenious procedure known as the *method of Lagrangian multipliers* (see Epstein,

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[†] That this is so can be seen by supposing one of the terms, e.g., $L_2 \alpha_2$, to be different from zero. Since by supposition, Eq. (131) must be true for any set $d\alpha_1, \ldots, d\alpha_n$ it must be true for a set of the type $d\alpha_1 = \ldots d\alpha_3 = 0$, $d\alpha_2 \neq 0$. Under this circumstance Eq. (131) reduces to the single term $L_2 \alpha_2 d\alpha_2 = 0$, which cannot be true unless $L_2 \alpha_2 = 0$. Since this argument can be made with respect to any term of the sum it is obvious that $L_j \alpha_j = 0$ for $j = 1, \ldots, n$.

1937, pp. 97–99, for a more extensive discussion of their theory and for application to more general and complex systems of equations than the one now under discussion). Suppose that Eq. (130) is multiplied by some number λ and subtracted from Eq. (131). One would then obtain the relation

$$\sum_{j=1}^{n}(L_j\alpha_j-\lambda)d\alpha_j=0 \tag{132}$$

Now it is clear that one may choose any arbitrary set of variations $d\alpha_2, \dots , d\alpha_n$ and always satisfy Eq. (130) by choosing

$$d\alpha_1=-\sum_{j=2}^{n}d\alpha_j$$

If λ is chosen to be equal to $L_1\alpha_1$ then

$$(L_1\alpha_1-\lambda)=0 \tag{133}$$

and

$$\sum_{j=2}^{n}(L_j\alpha_j-\lambda)d\alpha_j=0 \tag{134}$$

However, since the set $d\alpha_2, \dots , d\alpha_n$ is *completely* arbitrary, in order for Eq. (134) to be true it must be that $(L_j\alpha_j-\lambda)=0$ for $j=2, \dots , n$. Therefore

$$\lambda=L_1\alpha_1=L_2\alpha_2=\dots=L_n\alpha_n$$

and because of Eq. (127),

$$u_1=u_2=\dots=u_n$$

which is just what one obtains by requiring that $dR_j/dt=0$ for $j=1, \dots , n+1$. Thus, in this case, the Prigogine minimum entropy production principle gives the same result as the more conventional requirement that the concentrations be time-independent for the steady state.

The method of Lagrangian multipliers illustrated in this simple example is a powerful general method for handling the situation in which one has some function, e.g., entropy, free energy, irreversible entropy production, in which the conditions of its maximum or minimum value are being sought with respect to parameters on which there are equations of constraint. For example, in the case of Eq. (9) one might desire to examine the equilibrium configuration of a system in which there were a number of equations of constraint relating the mole numbers, generalized displacements, etc. An excellent discussion and many examples of this manner of treatment of classical thermodynamic problems may be found in Epstein (1937).

VII. The Time-Dependent State

In the discussion throughout the chapter it has been assumed that the reader has some previous intuitive notions regarding the terms "equilibrium," "steady state" and "stationary state". Aside from those places in which specific mention has been made of notions of stationarity at odds with those of the present writers, the term *steady state* means that condition of the system in which the composition and spatial distribution of matter and charge are independent of time. Since the systems of interest are spatially uniform with respect to temperature and pressure there is no need to be concerned with these variables at the moment.

When some irreversible process or set of processes is first initiated in a system, the system does not pass immediately into a stationary state but instead exhibits some sequence of temporal changes in its composition and spatial distribution of its constituents. The set of values of the variables necessary to describe this initial condition of the system is called the *initial conditions.* One can then concern oneself with the temporal transition to the steady state, or the question of whether such a steady state even exists for the system of interest. One may also consider a system that has been disturbed from its steady state and investigate the time-course of the recovery to the steady state, or whether such recovery occurs at all. In general these problems tend to become difficult for systems of any complexity, and it must be asserted at the outset that the theory of such matters is still largely unexplored.

Not all chemical systems possess a steady state other than that in which the various reactions of the system are in equilibrium, i.e. all the rates are zero. Consider a system of reactions in which a certain number of the reaction intermediates are not free to escape from the cell. For example, it is generally conceded that the cell walls are relatively impermeable to substances such as adenosine triphosphate and hexose phosphates. For simplicity let us consider the reactions and participating substances to be spatially uniform in the cell. Using the notation already introduced previously, one can then write the rate of change of the concentration of each of these intermediates in terms of the rates of reaction u_j, $j = 1, \ldots, n$, with the understanding that these refer to the rate per unit volume. Thus

$$\frac{dC_k}{dt} = \sum_{j=1}^{n} m_{kj} u_j \qquad k = 1, \dots, M \tag{135}$$

where there are n reactions and M intermediates incapable of entry or exit from the system. Now for the system to have a steady state it is necessary, by definition, to require that

$$dC_k/dt = 0 \qquad \text{for} \qquad k = 1, \dots, M$$

This means that there must exist some set of rates $u_1, \dots, u_n$ such that the system of M equations in the n u's

$$0 = \sum_{j=1}^{n} m_{kj} u_j \qquad k = 1, \dots, M \tag{136}$$

can be satisfied. It is clear that it can always be satisfied for $u_1 = u_2 = \dots = u_n = 0$; however, this means that the system is in equilibrium, and this is not very interesting from the point of view of metabolism.

By the rules of elementary algebra one knows that one can perform the following two manipulations on a system of equations such as Eq. (136) without altering the set of $u_1, \dots, u_n$ satisfying the system.

1. The relative position of any two equations can be interchanged.
2. Any equation of the system can be replaced by the equation resulting from multiplying some other equation of the system by an arbitrary number and adding it to the equation to be replaced, e.g., if

$$\sum_{j=1}^{n} m_{j1} u_j = 0, \quad \sum_{j=1}^{n} m_{j2} u_j = 0, \quad \dots, \quad \sum_{j=1}^{n} m_{jM} u_j = 0$$

are the equations of the system, then these can be replaced by the system of equations

$$\sum_{j=1}^{n} (m_{j1} + \lambda m_{j2}) u_j = 0, \quad \sum_{j=1}^{n} m_{j2} u_j = 0, \quad \dots, \quad \sum_{j=1}^{n} m_{jM} u_j = 0$$

where λ is an arbitrary number. If all the coefficients of the u's in an equation are zero, then that equation is eliminated from the system of equations. It may be possible to eliminate some equations by such type 2 manipulations on the system and thereby reduce the number of equations in the resulting system. There will be some minimum number of equations, however, such that no type 2 manipulation will result in any further eliminations or reduction of the number of equations in the resulting system. This minimum number of equations to which the system can be reduced is called the *rank* of the system. It can be demonstrated that the rank will never exceed the number of variables and can obviously not exceed the number of equations.

In order for there to exist a set of u's not all zero, satisfying Eq. (136), it is necessary that the rank be less than or equal to $n-1$.†

In the light of the generally prevailing intuitive feeling that metabolic systems be capable of nonequilibrium steady-state operation one must come to the conclusion that, for a set of conceivable reactions to constitute an admissible description of a real metabolic system, the stoichiometric coefficients of nondiffusible intermediates in the set of postulated reactions must fulfill the above requirements for the existence of a nonequilibrium steady state solution of Eq. (135).

When a reaction system capable of steady-state operation is displaced from the steady state one might ask whether it will restore itself to the same steady state, undergo transition to some new steady state, etc. Such questions, as might be anticipated, are highly relevant to the interpretation of the various rhythmic and periodic phenomena or the intrinsic homeostatic stability properties exhibited by metabolic systems. Although no general answer can be given there are some things that can be said along these lines for certain classes of reaction systems.

In the case of a number of reactions the rate can be represented as linear in one of the reactants. This does not mean that the reaction is monomolecular, but it does mean that it formally obeys first-order kinetics. Thus, for example, the rate of incorporation of an isotope present in trace amounts and the hydrolysis of sucrose in an aqueous medium would exhibit such first-order kinetics. Other reactions might be so represented. Consider a system of the sort

$$R_1+R_2 \rightleftarrows R_3+R_4$$
$$R_3+R_5 \rightleftarrows R_6+R_7$$

If the concentrations of R_1, R_4 and R_7 are held constant and R_2, R_3, and R_6 allowed to vary, then the rates of the two reactions can be looked upon as linear in the variable concentrations of R_2, R_3, and R_6. The terminology, of course, bears no relation to the physical linearity of the system and might equally apply to straight chain, branched or cyclic reaction sequences. To be exact, it means that for a cellular system in which the concentrations $C_1, \dots, C_n$ of n constituents are variable that

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† In mathematical terminology this means that the number of non-zero elements in the diagonal of the Hermitian form of the $M \times n$ matrix $[m_{kj}]$ must be less than or equal to $n-1$. A further discussion of such matters can be found in Macduffee (1943) or Dickson (1939).

$$V\frac{dC_k}{dt} = \sum_{j=1}^{n} A_{kj}C_j + Ah_k(C_k^0 - C_k) \qquad k = 1, \ldots, n \tag{137}$$

where A is the area of the cell surface, h_k the permeability constant for the kth substance, and C_k^0 the external concentration, assumed to be held constant for the kth substance. The coefficients A_{kj} are constants.

Hearon (1953) has demonstrated *for reaction systems describable by a set of differential equations of the form of Eq. (137) that the conservation of mass and the principle of detailed balancing imply that the C_k cannot be periodic functions of time.* It can further be demonstrated that *if the system is displaced from the stationary state and "released" then C_k will overshoot its stationary state value n times at most.*

It is evident from the conclusions of Hearon that one cannot hope to explain periodic phenomena in metabolic systems by any system of rate laws linear in the concentrations. An example of a nonlinear system exhibiting unstable properties that can lead to oscillatory behavior is discussed by Moore (1949), but whether or not this system corresponds with the kinetics of any metabolic system is unknown.

List of Principal Symbols

A	Area
C_k	Concentration of the kth constituent
D_k	Diffusion coefficient of the kth constituent
D_{ik}	Generalized diffusion coefficient (Onsager) relating the flux $\mathbf{J}_i$ to the concentration gradient ∇C_k of the kth constituent
d—	Total differential change in — where — is a variable of state
$đ$—	Differential change in — where — is not a variable of state
E	Internal energy
e	Naperian log base
F	Gibbs free energy
H	Heat content or enthalpy
$\mathbf{I}$	Flux of electric charge or current density (a vector quantity)
$\mathbf{i}, \mathbf{j}, \mathbf{k}$	Unit vectors oriented along the directions of the x, y, and z axes of a Cartesian reference frame in three-dimensional space
$\mathbf{J}_k$	Flux (a vector) of the kth constituent
K	Solubility product ; dissociation constant of a molecular complex
K_1, K_2, K_3	Equilibrium constants for the 1st, 2nd, 3rd reactions
k_1, k_2, etc.	Rate constants
L	A generalized displacement ; coefficient of proportionality between the driving force and the flux
L_k	Coefficient of proportionality between the driving force and the rate of the kth process
L_{ik}	Phenomenological coefficient relating the ith flux to the kth force
$\ln C$	Natural (Naperian) logarithm of C
m_k	Stoichiometric coefficient of the kth substance in a reaction, assigned a negative value if the kth substance is a reactant and a positive value if it is a product

m_{kj}	Stoichiometric coefficient of the kth substance in the jth reaction. negative for reactants, positive for products, and zero for nonparticipant in the jth reaction
n_k	Number of moles of the kth constituent
n_{kj}	Number of moles of the kth constituent in the jth phase
$d_e n_k$	Differential change in the number of moles of the kth constituent added to the system from the environment
N_a	Avogadro's number, i.e., the number of molecules in a mole of material
P	Pressure
$\text{đ}Q$	Infinitesimal increment of heat added to the system
$\text{đ}Q^*$	Infinitesimal increment of uncompensated (deDonder) heat
q_k	The rate of production (per unit volume) of the kth constituent
dr	An infinitesimal displacement (a vector) in three-dimensional space
R	The gas constant
S	Entropy
$d_i S$	Differential change in entropy due to processes internal to the system, i.e., the irreversible entropy change
$d_e S$	Differential change in the entropy of the system due to a reversible exchange between the system and the environment
t	Time
T	Absolute temperature
u_j	Rate of the jth reaction
$\mathbf{v}_k$	The average velocity (a vector) of the molecules of the kth constituent
v_k	The partial molar volume of the kth constituent
V	The volume of the system
$\text{đ}W$	Infinitesimal increment of work done by the system
$\text{đ}W'$	Infinitesimal increment of nonpressure volume work done by the system
$\mathbf{X}_k$	Force (a vector) on a mole of the kth constituent
x, y, z	Cartesian coordinates defining a point in three-dimensional space
z_k	Charge valence of the kth ionic species
α	The affinity (deDonder) of a reaction
α_j	The affinity (deDonder) of the jth reaction
γ	Surface tension ; Activity coefficient
Γ	The Faraday constant, i.e., the electric charge possessed by one gram equivalent of ions
Δ—	Used to denote a noninfinitesimal difference in the quantity —
δ	Thickness of the surface layer
λ	An arbitrary number used as a multiplier
μ_k	Chemical potential or partial molar free energy of the kth constituent
μ_{kj}	Chemical potential of the kth constituent in the jth phase
$\overset{0}{\mu}_k$	Standard chemical potential of the kth constituent
$\overset{0}{\mu}_{kj}$	Standard chemical potential of the kth constituent in the jth phase
ξ	Degree of advancement (deDonder) of a single reaction
ξ_j	Degree of advancement (deDonder) of the jth reaction
χ_k	Mole fraction of the kth constituent
Ω_k	Mobility of the kth constituent
$\nabla\phi$	The gradient (a vector) of the scalar function ϕ
$\nabla\cdot\mathbf{u}$	The divergence (a scalar) of the vector function $\mathbf{u}$
$\mathbf{u}\cdot\mathbf{v}$	The inner product (a scalar), sometimes called the " dot " product, of two vectors $\mathbf{u}$ and $\mathbf{v}$

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CHAPTER 3

Cybernetic Aspects of Homeostasis†

STANFORD GOLDMAN

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I. Introduction

Homeostasis is the maintenance of a prescribed internal environment in the body of an organism, in spite of wide fluctuations in its activity and external environment. The maintenance of a relatively constant body temperature and a relatively constant blood sugar level are examples of homeostasis. Many fundamental ideas concerning homeostasis go back at least to Claude Bernard, but the term *homeostasis* was introduced by Walter B. Cannon about 30 years ago. Cannon carried on extensive experimental investigations in this field and wrote on its philosophy and implications.

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† This chapter is based upon research done by the author under the sponsorship of the Office of Naval Research.

About 1932, when Cannon published his " The Wisdom of the Body," H. S. Black (1934) developed the general theory of the use of negative feedback for stabilization purposes.† Black was led to the development of his theory by the attempt to stabilize amplification in transcontinental telephone lines. Further development of the theory of negative feedback for use in control systems was stimulated by the rapid development of automatic control equipment, gunfire control equipment, and guided missiles. The theory of control systems, usually under the name of servo theory,‡ is now a well-established branch of engineering.

Another new branch of learning with which we shall be concerned is the quantitative theory of the transmission of information, sometimes called communication theory. The classical work in this field has been done by Hartley (1928), Wiener (1948), and especially Shannon. Shannon's development of communication theory was first published in 1948–1949.

Some of the ideas on the use of feedback for control purposes in physiology may be found in the work of Cannon and earlier physiologists. By the early 1940's, some physiologists even used the terminology of servo theory in dealing with biological control systems. In 1948 the mathematician Norbert Wiener published his book " Cybernetics." In this book the name " cybernetics " is used to describe the entire field of control and communication theory, whether in the machine or in the animal. The word is derived from the Greek word meaning " steersman."

In the present article we shall develop *ab initio* the principles of feedback theory, servo theory, and information theory and shall try to see what light they can throw on the study of homeostasis.

II. Control System Theory

A. Introductory Ideas

A control system is a system in which a stimulus is translated into a response. In other words, the activity of the control system causes a particular stimulus to bring about a particular effect. For example, the steering system of an automobile is a control transducer§ which makes the automobile turn in response to the turning of the steering wheel by the driver. If, in turn, something comes back from the response to alter the control stimulus, this process is called feedback. If a man drives an automobile, the position of the automobile is " fed back " to the driver via his eyes. This new information is used by the driver in deciding whether and how to turn the steering wheel. A schematic diagram of the operation of a control system is shown in Fig. 1.

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† The use of negative feedback in amplifiers for other purposes, such as neutralization, is older. It is of interest to note that Wiener (1948) states that the first significant paper on feedback mechanisms is an article on *governors* published by J. C. Maxwell in 1868. This paper of Maxwell's is well worth reading even today.

‡ A servo system is a control system based on the use of feedback.

§ A device which translates a signal from one form into another is called a transducer.

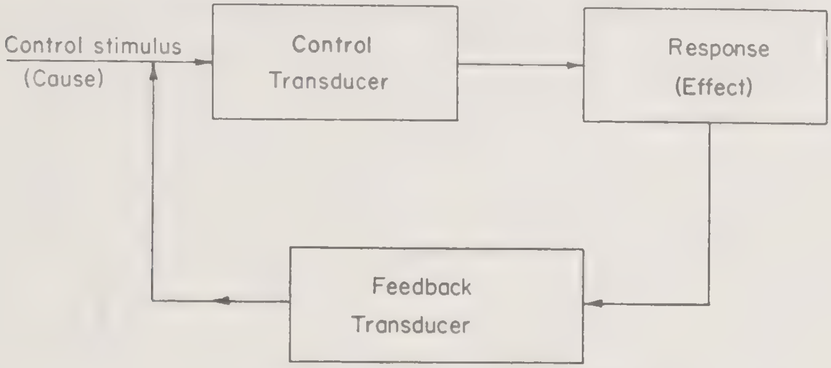

FIG. 1. Schematic diagram of a control system.

If, in the operation of a control system, nothing comes back from the output (the response) to affect the input (the stimulus), then the system is described as an " open cycle system". On the other hand, a control system with feedback as described above is called a " closed cycle system." Figure 1 illustrates a closed cycle control system. For example, suppose a hostess drops a block of ice into a punch bowl to cool the punch, but makes no attempt to reach a special temperature. This is an example of an open cycle control system. If, on the other hand, the hostess tests the temperature of the punch periodically and adds ice whenever necessary to keep the punch as cold as desired, the system has become a closed cycle system.

Almost all biological control systems are closed cycle systems. Closed cycle control systems are called *servo* systems. In such a system the difference between the desired and the actual output is called the *error*. In general, a servo system is actuated by an *error signal* which is used to cause an action which will reduce the error. This process is called *negative feedback* (of the error).

Cases of negative feedback in the human body are abundant. For example :†

1. Low blood sugar causes hunger, which causes eating, which causes restoration of the blood sugar level. In this case, the error is the difference between the low sugar value and the normal, while hunger represents a negative feedback of the error signal, since its ultimate effect is to cause reduction of the error.

† These examples are simplified descriptions of phenomena which are actually quite complex. It is hoped, however, that they will serve to illustrate negative feedback.

2. High intensity of illumination on the retina causes contraction of the pupil of the eye, which in turn reduces the illumination on the retina toward an optimum value. On the other hand, low intensity of illumination on the retina causes dilation of the pupil, which in turn increases the illumination on the retina toward an optimum value. The error signal in these cases is the signal which is fed from the retina to the pupil. There is negative feedback of the error in both cases since the effect of the feedback is to reduce the error.

We can classify control systems into different types, five of which are listed below. The type of control system used is important because it affects the speed of control, the accuracy of control, and the versatility and stability of the system.

Type 1. Directed correction. Constant (+ or −) " off " or " on " action to reduce error. Example : thermostat with off-or-on heater. This is the crudest type of control.

Type 2. Proportional control. Action proportional to error. Example : thermostat with heating current proportional to the temperature deviation. This is ordinarily faster and more accurate than Type 1.

Type 3. Proportional plus derivative control. Action proportional to the rate of change of the error is used to prevent overshoot (i.e., error in the opposite direction). This is used in gunfire control to anticipate the location of the target on the basis of its present velocity.†

Type 4. The foregoing plus *integral control*. Action proportional to the time integral of the error is introduced. This increases the controlling action if the system is failing to reduce the error sufficiently. Integral control increases the accuracy of control and reduces any tendency of the system to drift into error.†

Type 5. Control due to anticipation of future events. This is the type of control used by the driver of an automobile when he sees a turn in the road coming ahead.

These types of control are all illustrated in the following quotation from Ivan Getting (1947) concerning servo systems in human behavior :

" Actually, human physical motor behavior is largely controlled as a servo system. A person reaches for a saltcellar. He judges the distance between his hand and the saltcellar. The distance is the ' error ' in the position of his hands. Through his nervous system and subconscious mind this error is used to control muscular motion, the power being derived from the muscular system. As the distance decreases, derivative control is brought into play through subconscious habit, and overshooting of the hand is prevented. A more illustrative example is the process of driving a car. A person who is just learning to drive generally keeps the car on a road by fixing his attention on the edge of the road and comparing the location of the edge of the road with some object on the car, such as the hood cap. If this distance is too small, the learner reacts by turning the steering wheel to the left ; if it gets too large, he reacts by turning the steering wheel to the right. It is characteristic of the learner that his driving consists of a continuous series of oscillations about the desired position. The more carefully he drives, that is, the greater his concentration, the higher will be the frequency of his oscillations and the smaller the amplitude of his errors. As the driver improves, he introduces anticipation, in the form of derivative control. In this condition a driver takes into consideration the rate at which he is approaching his correct distance from the edge of the road, or, what is equivalent, he notices the angle between the direction of car travel and the direction of the road. His control on the steering wheel is then a combination of displacement control and derivative control. His oscillations become long or nonexistent, and his errors smaller.

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† A more detailed discussion of Types 3 and 4 is given in Section II, C.

"So long as the road is straight, a driver of this type, acting as a servo-mechanism, performs tolerably well. However, additional factors come into play as he approaches a bend in the road. Chief among these is the displacement error resulting from the tendency of the operator to go straight. The error due to continuous uniform curvature of the road can be taken out by essentially establishing a new zero position for the steering wheel. A driver performing in this manner exhibits 'integral control'. Actually a human being is not a simple mechanism, and he has available in this instance information of other types. His driving is a complicated combination of proportional, derivative, and integral control, mixed with nonlinear elements and knowledge of the direction in which the road is going to turn. This foreknowledge is sometimes referred to as anticipation and is sometimes confused with derivative control. This example nevertheless serves to illustrate the basis of servo mechanisms in general. The power to be controlled in this case was derived from the engine of the car. The input to the system was the actual path of the road ; the output was the position of the car ; and the error mechanism in which the output and the input were compared was the human operator."

Control systems sometimes get out of order, and it is important to know the types of trouble to which they are subject. A servo system is expected to use negative feedback to reduce the error. However, in faulty operation the feedback can become positive and will increase the error. If the control action is strong in such a case, the error may increase tremendously and the whole system will become unstable. To give a physiological example, spastic activity in a human being may arise in this way.

B. Elementary Mathematical Treatment of the Reduction of Errors by the Use of Feedback†

1. *Feedback of Signal to the Input*

In this section we shall calculate the amount of error reduction achieved by the use of feedback in the case when a signal derived from the error is fed back and added to the input signal. This is the type of system most commonly used in electrotechnical practice, and it also occurs frequently in biology.

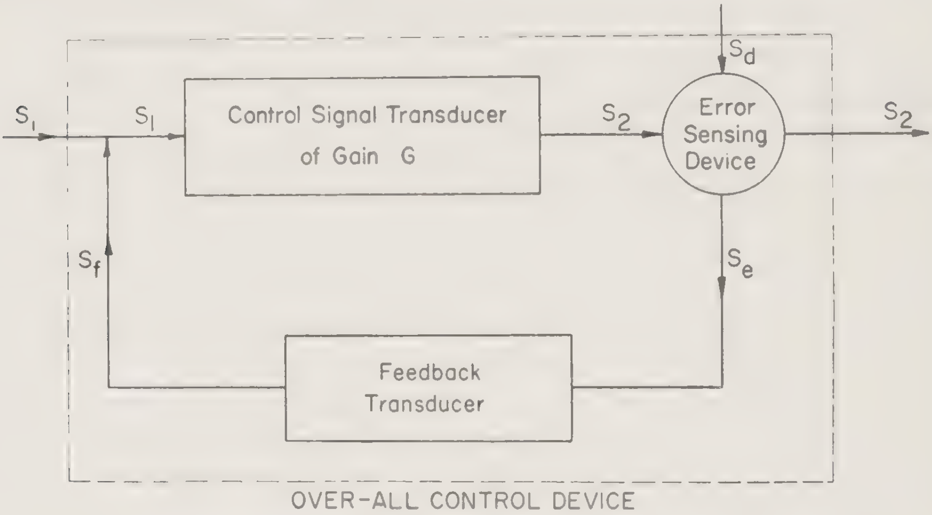

FIG. 2. Control system in which feedback signal is added to the input.

A diagram of the system operation is shown in Fig. 2. An input signal S_1 into a control device causes an output signal or response S_2. Let us use the following terminology :

S_d = desired output signal (or desired effect)
S_i = input signal to over-all control device
S_2 = actual output signal
S_e = error = $S_2 - S_d$
S_f = signal fed back to be added to the input
S_1 = input to control signal transducer = $S_i + S_f$
G = S_2/S_1 = amplification factor of control signal transducer
B = feedback factor = S_f/S_e

---

† In Section II, B, we shall consider only the case in which the error correction effect of the feedback action is instantaneous. This does not mean that the error is completely eliminated, but only that whatever error reduction effect is achieved is obtained immediately. In Section II, C we shall consider the case in which the error reduction effect of the feedback signal is not instantaneous.

From the foregoing terminology, it follows that the error which would be present in the absence of feedback, which we may call S_{eo}, is

$$S_{eo}=GS_i-S_d \tag{1}$$

Let us next consider what happens in the presence of feedback. In this case we have

$$\begin{aligned} S_f=BS_e&=B(S_2-S_d)=B(GS_1-S_d)\\ &=B[G(S_i+S_f)-S_d] \end{aligned} \tag{2}$$

Therefore

$$S_f[1-BG]=B[GS_i-S_d]=BS_{eo} \tag{3}$$

Thus

$$S_f=\frac{B}{1-BG}S_{eo} \tag{4}$$

and the error is

$$S_e=S_2-S_d=GS_1-S_d=G(S_i+S_f)-S_d \tag{5}$$

$$=GS_f+S_{eo}=\frac{BG}{1-BG}S_{eo}+S_{eo}=\frac{1}{1-BG}S_{eo}$$

Consequently feedback causes the error to be multiplied by the factor

$$\frac{1}{1-BG}.$$

In the case of negative feedback, BG is a negative quantity, so that the above factor is less than one and the error is reduced. In many practical cases, the value BG may be as great as (-100) so that feedback reduces the error to less than one percent of what it would be without feedback.

2. *Feedback to Change Network Parameters*

We will next consider the case where the feedback signal, instead of being added to the input signal, is used to change the operating characteristics of the control transducer. In servo terminology, we would say that the feedback signal is used to change the network parameters. This is shown on Fig. 3. This type of control operation is very common in biological organisms.

We shall use the same meanings for symbols in the analysis of this system as used in Section II, B, 1. The action of the control transducer

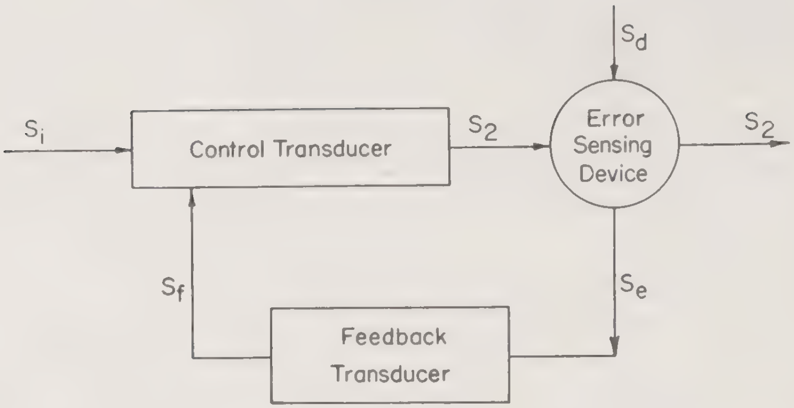

FIG. 3. Control system in which feedback changes network parameters.

is described mathematically by the the symbol G. Since G varies with the feedback signal S_f in this case, we can express the relation between G and S_f as a Maclaurin series.† Thus

$$G = G_0 + aS_f + bS_f^2 + cS_f^3 + \dots \tag{6}$$

As a first approximation for our analysis, we will accordingly use the formula

$$G = G_0 + aS_f \tag{7}$$

For the present case, we now have

$$S_2 = GS_i = (G_0 + aS_f)S_i \tag{8}$$

$$S_{eo} = G_0 S_i - S_d \tag{9}$$

$$S_e = S_2 - S_d \tag{10}$$

$$\begin{aligned} S_f &= BS_e = B(S_2 - S_d) = B[S_i(G_0 + aS_f) - S_d] \\ &= B(aS_f S_i + S_{eo}) \end{aligned} \tag{11}$$

Thus

$$S_f(1 - BaS_i) = BS_{eo} \tag{12}$$

or

$$S_f = \frac{B}{1 - BaS_i} S_{eo} \tag{13}$$

Finally

$$S_e = \frac{S_f}{B} = \left(\frac{1}{1 - BaS_i}\right) S_{eo} \tag{14}$$

---

† Maclaurin series are discussed in elementary texts on calculus, such as Sherwood and Taylor (1946, p. 397).

In this case then, the error reduction is described by the factor $1/(1 - BaS_i)$. Ordinarily the feedback is negative, so that Ba is a negative quantity.

3. *The Regulation of Concentration by Feedback, Using Storage Elements*

We will now consider what is essentially a special case of the type of system analysed in Section II, B, 1 and illustrated in Fig. 2. We shall consider the regulation of concentration by feedback operating on a storage system. Let us suppose that we have a solution in which we wish to keep the concentration of a particular solute at a desired value, even though various amounts of this solute may be added to or removed from the solution either by external action or perhaps by internal chemical reaction. In order to regulate the concentration, the solution is connected to a storage device where the solute of interest is stored, and the storage device can remove solute or add it to the solution. The storage device operates in response to the level of concentration of the solute. A schematic block diagram of the operation of this system is shown in Fig. 4.

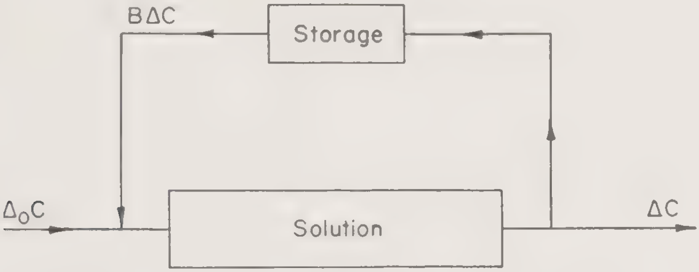

FIG. 4. A storage device as a feedback transducer.

Let us suppose that the solution in Fig. 4 actually has the desired concentration of the solute. Then any addition or removal of the solute of interest causes an undesired change in concentration. Let us now suppose that additional solute is externally added, so that its concentration would rise by an amount, $\Delta_0 C$, if the storage system did not respond. We may consider $\Delta_0 C$ to be S_i for this system as well as S_{eo} in the terminology of Section II, B, 1. Thus

$$S_i = \Delta_0 C = S_{eo} \tag{15}$$

Let us suppose that ΔC is the actual rise in concentration, including both effects of the storage system and the solute externally added. Then we may write

$$S_2 = \Delta C = S_e \tag{16}$$

We note in passing that in this case we have

$$S_d = \text{desired deviation from desired concentration} = 0 \tag{17}$$

In the absence of feedback, that is, in the absence of action by the storage device (indicated by primes on the symbols), we would have

$$\Delta' C = \Delta_0 C \tag{18}$$

so that

$$S_2' = S_i = S_1' \tag{19}$$

We may therefore conclude that

$$G = \frac{S_2'}{S_1'} = 1 \tag{20}$$

Now
$$S_f = BS_e = B\Delta C \tag{21}$$

where $B\Delta C$ is the amount of solute added to the solution by the storage system in response to the deviation ΔC.
Thus

$$\Delta_0 C + B\Delta C = S_1 = S_2 = \Delta C \tag{22}$$

Assuming that the feedback is negative, B must be a negative quantity. Let us use β for the magnitude of B. Then

$$\frac{S_e}{S_{eo}} = \frac{\Delta C}{\Delta_0 C} = \frac{1}{1 - BG} = \frac{1}{1 + \beta} \tag{23}$$

The ratio S_e/S_{eo} is the factor by which the regulating system reduces the deviation from the desired concentration. The foregoing analysis tells us that if a rise in concentration of ΔC will cause a storage of solute equivalent in amount to $\beta\Delta C$, then the regulating system will reduce deviations from the desired concentration by the factor $1/(1+\beta)$. Another way of stating this is that if enough solute is added to raise the concentration by an amount $\Delta_0 C$ in the absence of feedback, then because of feedback the fraction $\beta/(1+\beta)$ of the added solute goes into storage and the fraction $1/(1+\beta)$ causes an actual rise in concentration. We note in passing that this analysis assumes that the storage system responds instantaneously to the concentration.

The foregoing system is of interest because of its similarity to the regulation of desired solutes in the blood. The analogy is by no means complete, however, because the storage systems in the body usually do not act instantaneously.

4. *A Buffer Solution as a Feedback Mechanism*

A buffer solution is one important example of a system for which the analysis of Section II, B, 3 will serve as an accurate model because its feedback operation is essentially instantaneous.

Let us consider an aqueous buffer solution whose solute consists of a weakly dissociated acid, HA (say acetic acid), and a completely dissociated salt, BA (say sodium acetate) with the same acid radical. The solution will therefore contain the ions H^+, OH^-, A^-, and B^+, as well as molecules HA and H_2O. We will assume, for simplicity, that BA and BOH are completely dissociated. If additional H ions are added to this solution, for example by dissolving a relatively small amount of a completely dissociated acid such as HCl, most of the additional H ions will be removed by combination with A ions to form weakly dissociated HA. A very small amount of the additional H ions will also be removed by combination with OH ions to form water. In effect, then, we may consider the buffer solution to react by putting most of the H ions into storage as HA.

If additional OH ions are added to the solution, for example by adding a small amount of a completely dissociated base (say NaOH), most of the additional OH ions will be removed by combination with H ions to form water. We may consider this process as putting the OH ions into storage as water. It is probably more instructive, however, to consider the added OH ions as a negative quantity of H ions which is eliminated by taking positive H ions out of HA storage.

In order to determine the effectiveness of the buffer action, let us formulate the action in terms of the formulas of Section II, B, 3. According to the law of mass action, we have

$$\frac{[H^+][A^-]}{[HA]} = K \tag{24}$$

where any quantity in brackets stands for the molar concentration of that quantity and the solutions are sufficiently dilute so that the activity coefficients may be considered unity. By differentiation of Eq. (24) we can see what change is caused in the other concentrations when one of the concentrations in Eq. (24) is varied by a small amount.

Thus

$$\Delta[\mathrm{H}^+]=\frac{K}{[A^-]}\Delta[\mathrm{H}A]-\frac{K[\mathrm{H}A]}{[A^-]^2}\Delta[A^-] \tag{25}$$

In Eq. (25), $\Delta[\mathrm{H}^+]$ is the actual change in concentration of H ions in the solution. Let us write $\Delta_0[\mathrm{H}^+]$ for the equivalent change in H-ion concentration which would occur if no buffer action took place. Then

$$\Delta_0[\mathrm{H}^+]=\Delta[\mathrm{H}^+]+\Delta[\mathrm{H}A]+\Delta[\mathrm{H_2O}] \tag{26}$$

where the terms on the right are the changes in concentration resulting from the addition of the acid when the buffer action does take place. Furthermore,

$$\Delta[\mathrm{H}A]=-\Delta[A^-] \tag{27}$$

It is the purpose of our analysis to find the ratio $\Delta[\mathrm{H}^+]/\Delta_0[\mathrm{H}^+]$, which is equivalent to S_e/S_{eo} in Eq. (23). For this reason we will now try to find the values of $\Delta[\mathrm{H}A]$ and $\Delta[\mathrm{H_2O}]$ in terms of $\Delta[\mathrm{H}^+]$, and then substitute these into Eq. (26). Inserting Eq. (27) into Eq. (25) and using Eq. (24), we have

$$\begin{aligned}\Delta[\mathrm{H}^+]&=\frac{K}{[A^-]}\Delta[\mathrm{H}A]+\frac{K[\mathrm{H}A]}{[A^-]^2}\Delta[\mathrm{H}A]\\&=\left(\frac{[\mathrm{H}^+]}{[\mathrm{H}A]}+\frac{[\mathrm{H}^+]}{[A^-]}\right)\Delta[\mathrm{H}A]\end{aligned} \tag{28}$$

Next, to find the value of $\Delta[\mathrm{H_2O}]$ we start with the dissociation equation of water

$$\frac{[\mathrm{H}^+][\mathrm{OH}^-]}{[\mathrm{H_2O}]}=K_w \tag{29}$$

and we also note that

$$\Delta[\mathrm{H_2O}]=-\Delta[\mathrm{OH}^-] \tag{30}$$

Differentiating Eq. (29) we then have,

$$\begin{aligned}\Delta[\mathrm{H}^+]&=\frac{K_w}{[\mathrm{OH}^-]}\Delta[\mathrm{H_2O}]-K_w\frac{[\mathrm{H_2O}]}{[\mathrm{OH}^-]^2}\Delta[\mathrm{OH}^-]\\&=\frac{K_w}{[\mathrm{OH}^-]}\Delta[\mathrm{H_2O}]+K_w\frac{[\mathrm{H_2O}]}{[\mathrm{OH}^-]^2}\Delta[\mathrm{H_2O}]\end{aligned}$$

$$= \frac{K_w}{[OH^-]}\left(1 + \frac{[H_2O]}{[OH^-]}\right) \Delta[H_2O]$$

$$= \frac{K_w}{[OH^-]}\frac{[H_2O]}{[OH^-]} \Delta[H_2O], \text{ approximately}$$

$$= \frac{[H^+]}{[OH^-]} \Delta[H_2O] \tag{31}$$

Substituting Eqs. (31) and (28) into (26) we have finally

$$\Delta_0[H^+] = \Delta[H^+] + \Delta[HA] + \Delta[H_2O]$$

$$= \Delta[H^+]\left(1 + \frac{1}{([H^+]/[HA]) + ([H^+]/[A^-])} + \frac{[OH^-]}{[H^+]}\right) \tag{32}$$

Equation (32) indicates that when $[OH^-]$ and $[H^+]$ are of about the same order of magnitude, most of $\Delta_0[H^+]$ goes into storage as HA. For a more basic solution, the amount of storage as H_2O would increase. Equation (32) gives the quantitative value of the effectiveness of the buffer action for added $[H^+]$. Comparing Eq. (32) with Eq. (23), we see that the value of the feedback factor β is

$$\beta = \frac{1}{([H^+]/[HA]) + ([H^+]/[A^-])} + \frac{[OH^-]}{[H^+]} \tag{33}$$

The analysis of the case in which a highly dissociated base is added can be treated in a generally similar manner.

C. Special Topics for Consideration

1. *Speed and Accuracy, Derivative and Integral Control, Use of the Past in Anticipation of the Future*

The systems considered in Section II, B were all analyzed under conditions using proportional control and instantaneous operation. The analysis illustrates the mechanism of the reduction of errors by feedback. Furthermore, it approximates the actual situation in cases such as those of the buffer solutions where the control is essentially proportional and feedback takes place practically instantaneously. In many feedback systems, however, errors are not corrected instantaneously, and controls other than proportional are often used. We shall now consider certain aspects of these more general cases. We shall not, however attempt a mathematical analysis.†

† The reader who wishes to see the mathematical analysis may consult a standard text on servo theory, such as that of Truxal (1955).

Consider the diagram in Fig. 5. This shows how the error in a hypothetical servo system would vary with time under various conditions of operation. Let us assume that there is an error ϵ, introduced at time t_1, and let us first of all assume that we have only proportional control. For a certain ratio of feedback signal to error, we might show a gradual decrease in error as shown in Curve A. If, however, we used a larger ratio of feedback to error, we could correct the error more quickly as shown by Curve B, but this might cause overshoot, to give error of opposite sign, as also illustrated in Curve B. It is, however, possible to get the increased speed of Curve B and yet avoid the overshoot by the introduction of derivative control. This is shown in the broken Curve C. Derivative control is the use in the feedback signal of a term determined by the rate of change of the error. This procedure has the effect of correcting the overshoot which might have been anticipated from the rate of decrease of the error if nothing had been done about it.

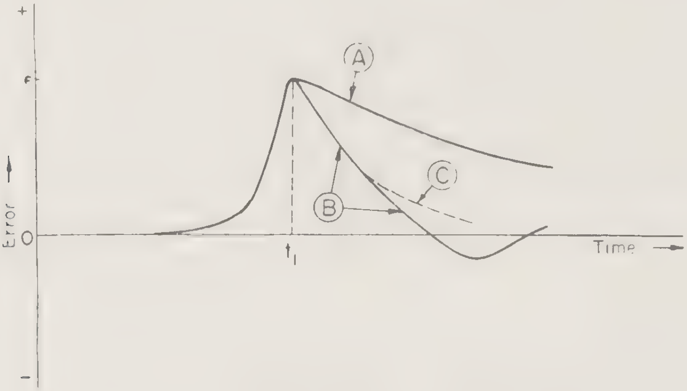

FIG. 5. Action of derivative control (shown by Curve C).

The value of derivative control is not limited to the correction of overshoot caused by the control system. If derivative control has been provided, it will come into play whenever the error is rising, with the net result that the feedback process starts to work on anticipated errors, so that the actual errors are smaller than they would otherwise have been.

In addition to speed of control, accuracy is also desirable. One way to obtain better accuracy is to increase the amount of feedback, as shown in Section II, B. However, a more efficient way for systems that do not operate instantaneously is to introduce integral control, as illustrated in Fig. 6. Let us suppose that an error introduced at t_1 decreases without integral control as shown in Curve A. The use of integral control is then shown in Curve B, where a term proportional to the time integral of the error is added to the feedback. The value of the time integral at any time t_2 is proportional to the area between the error curve and the time axis extending from t_1 to t_2. The longer the error has lasted, the greater the value of the integral, and therefore the greater its correction effect, even though the error has become small. Thus integral control improves the final accuracy attained and also increases the speed at which any particular accuracy is achieved.

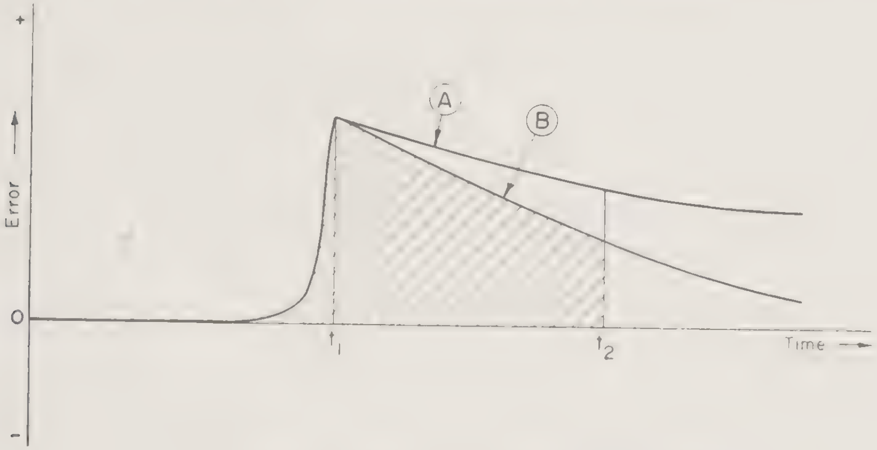

FIG. 6. Action of integral control (shown by Curve B).

It would be possible to refine the use of derivative and integral control by adding to the feedback terms proportional to higher derivatives and repeated integrals. This is done rarely, if ever, in man-made systems. However, something akin to it is employed in both man-made and biological systems, when knowledge of past history is used to predict future situations and to design or change system characteristics so as to give optimum operation under the expected conditions. In biological systems, this is called adaptation.

2. *The Use of Antagonists ; Instability*

In linear† electrical feedback systems, a negative error is in all respects similar to a positive error except for a change in sign, which means merely a change in direction of current flow. The same feedback mechanism which corrects a positive error will likewise correct a negative error. This ability to handle positive and negative errors with the same mechanism is not a universal property of feedback control systems. In biological systems, the mechanism to correct positive errors is frequently different from that correcting negative errors. In such a case, one mechanism may be called the antagonist of the other. In biochemical systems, it may be relatively easy to add one thing, but relatively difficult to remove it. It is therefore very convenient to be able to add an antagonist of an agent and thus obtain the equivalent effect of removing the agent. The use of a controlling agent plus its antagonist makes possible more rapid and accurate control, as the antagonist can prevent or cancel overshoot and in general balance out residual errors.

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† A linear system is one in which the response is exactly proportional to the stimulus, so that a plot of one versus the other is a straight line. In linear electrical systems, current is exactly proportional to voltage.

The use of antagonists, however, may also involve the danger of instability. Here " instability " has a special technical meaning, more or less equivalent to the terms " vicious cycle " or " runaway process ". Linear electrical feedback systems are also subject to this type of instability, but the antagonists in the latter case, while present mathematically, are somewhat more elusive. A discussion of instability in terms of antagonists actually clarifies the situation considerably and brings out certain ideas which are not obvious from the standard mathematical analysis,† even though the latter gives certain quantitative information which cannot be obtained by other means.

Figure 7 shows how an error may vary with time in a system which has a tendency toward instability. At time t_0, a given characteristic of the system falls below its desired level and a controlling agent starts to act to correct the error. Let us suppose that it takes the controlling agent approximately time T_1 to do its job. For simplicity in discussion, we have idealized the situation in Fig. 7 to show the entire action of the controlling agent as taking place precisely after a time T_1 has elapsed. Let us furthermore suppose that the action of the controlling agent is so strong that it overcorrects to cause an even greater error in the opposite direction.‡ At this juncture, the antagonist of the controlling agent comes into play. Let us suppose that the antagonist requires a time T_2 to act and that it is so strong as to overcontrol and to cause an even larger error in the opposite direction. After the time T_2 the original controlling agent comes into play again, this time to correct an error larger than the original error.§ As the process continues back and forth, the errors continue to grow in magnitude. Ultimately the growth stops because either the controlling agent or the antagonist is no longer able to cope effectively with the very large errors. The final state may be either an oscillation back and forth between large errors of opposite sign, or the system may be locked in a state of large error from which it cannot correct itself, because either the controlling agent or the antagonist cannot correct the final error. This is instability.[†]

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† A mathematical analysis of instability and oscillation in linear feedback systems may be found in Goldman (1949, Chapter 12).

‡ This would involve a feedback factor, β (Section II, B) of a magnitude greater than 2. Most well-controlled, stable systems actually have values of β much larger than 2.

§ It is not actually necessary that the error should grow in each half oscillation, as shown in Fig. 7, but only that it should grow in each full oscillation.

An examination of Fig. 7 in conjunction with the foregoing paragraph, shows that in order to avoid instability of the above depicted type in strongly controlled systems, the effect of both the controlling agent and the antagonist should start early, or more precisely, not too late. Then those parts of the action of the controlling agent and the antagonist which are supposed to cancel each other will occur simultaneously so that they actually do cancel instead of being regenerative. If this is accomplished, the antagonists work effectively to cancel the original error. In the case on instability, they are in effect acting against each other, instead of working against the original error.[‡]

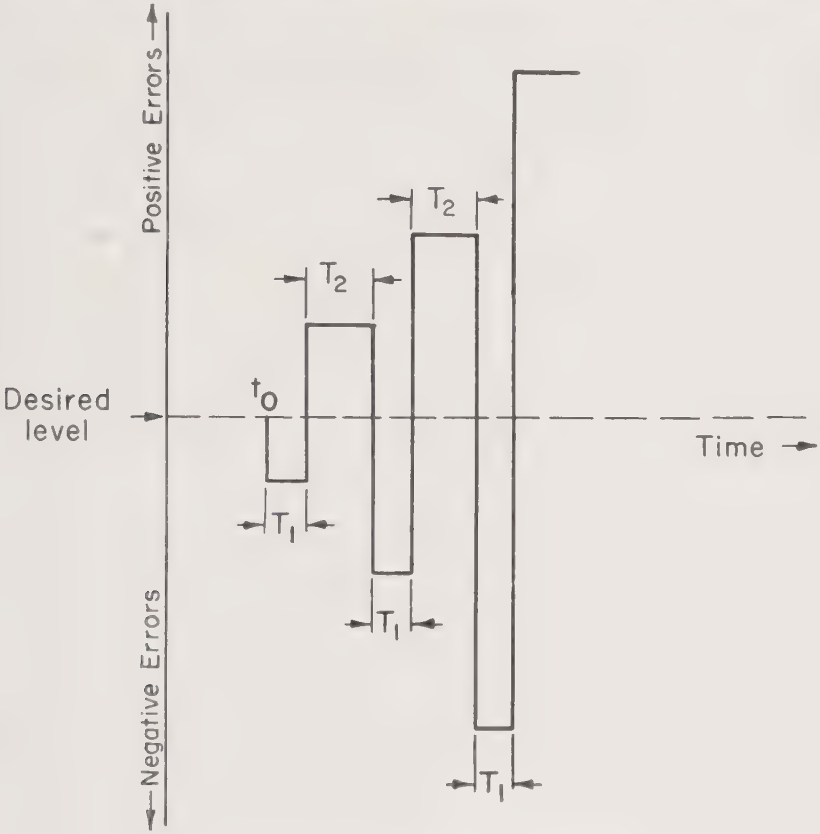

FIG. 7. Growth of error in case of instability.

[†] This same type of feedback system is actually put to use in the design of electronic oscillators and frequency generators.

[‡] In certain technical systems (constant frequency operation) derivative and integral control act antagonistically, i.e., 180 degrees out of phase. It is of interest to see whether this antagonism between the actions of derivative and integral control is exhibited elsewhere and to see what the apparent reason for the antagonism is in these other cases.

It is also possible for the action of an antagonist to start too early. If this occurs, the controlling agent becomes inefficient because so much of its effect is canceled. This can lead to a demand for an exorbitant amount of controlling agent (and antagonist) in order for regulation to be accomplished. It may happen that the source of supply of controlling agent cannot meet this demand or that it may be unduly taxed by the requirement.

We have in the foregoing paragraphs described instability in control systems as being related to timing errors and the action of antagonists. This is a very instructive point of view in studying instability problems. It should, however, be noted that similar instability effects may be due to errors of other types. The description in Section IV, B of a possible mechanism of edema will illustrate a case of instability due to errors other than timing errors and not involving an antagonist. In general, if due to a fault in a control device, the feedback increases the error instead of decreasing it and the error continues to build up repeatedly in going through the control cycle until some pathological quasi-steady-state is reached, the condition may be described as servo instability.

III. Information Theory

A. Introduction

Information theory is a quantitative theory of the transmission of information. Most of the technical development in this field has been of an advanced mathematical nature for the details of which the reader is referred elsewhere.† In the present section we shall outline the ideas and results of information theory using a minimum of mathematics. The way in which information theory is related to homeostasis will then be discussed in Section IV.

B. Alphabets, Messages, and the Exponential Law of Choice

We shall start our presentation of information theory by developing the basic ideas and formulas pertaining to alphabets, messages, and choice.

Suppose we have a message consisting of a sequence of symbols drawn from an alphabet of L symbols. Then it may be shown that the number of different messages which are possible using a sequence n symbols long is

$$[\text{Number of different possible messages}] = L^n \qquad (34)$$

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† See, for example, the book by Goldman (1953).

For example, the Arabic system of numbers has an alphabet of 10 symbols, namely, 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9. According to Eq. (34) there should be

$$L^n = 10^6 \tag{35}$$

different six-digit numbers from 000,000 to 999,999. This is, of course, clearly true.

The Arabic system of numbers is one of the rare instances in which all of the L^n different possible sequences may be used in the transmission of information. A more typical case is represented by simple written English, using only capital letters and no punctuation. In this case, there is an effective alphabet of 27 symbols, i.e., 26 letters and a word space. However, only those combinations of symbols are used which constitute English words. In effect, this means that the English language uses an expanded alphabet consisting of all the words in a complete English dictionary. However, it may be shown that in the latter case we still have :

$$\begin{aligned}&[\text{Number of different possible messages}\\&\qquad\text{using a sequence } n \text{ symbols long}] = (L')^n\end{aligned} \tag{36}$$

where L' is a number less than 27 (actually somewhat less than 10).†

An expanded alphabet, consisting of groups of symbols, such as the English dictionary described above, is called a *code*. The fact that many of the possible combinations of English letter symbols, such as AVGM and BRDW, are not accepted as words in the code is described as a *constraint* of the code. The fact that combinations of words which are not grammatically correct are also not allowed is another constraint of the code. If we include the effect of the grammatical constraints of English, Eq. (36) will still be true, only the value of L' is still further reduced. *Thus the number of different possible messages in a sequence in any given code increases exponentially with the number of symbols in the sequence.*

The constraints of a code may be described as *fixed constraints*, which means that they definitely rule out certain of the combinations of symbols included in Eq. (34). Let us next consider the case in which a *language* is expressed in terms of a code and meanings are assigned to the various allowed combinations of symbols in the code in order to transmit information. It may be shown that the mathematical effect of the language is to introduce *probability constraints* on the allowed combinations of symbols.† Probability constraints do not rule out any combination of symbols, but rather regulate the average occurrence of the symbols. It may be shown that Eq. (36) will *for sufficiently long messages* hold even in the presence of probability constraints, but the effect of the probability constraints will be to reduce L' still further. Thus in the English language, using the English dictionary and grammatical code, the value of L' in Eq. (36) is somewhere in the neighborhood of 2.

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† For the derivation of this and other formulas in this section, see the author's book cited on page 78.

The general conclusion to be drawn from the foregoing paragraphs—and one which is basic in information theory—is that the number of different messages of a given length in a language, i.e., in a medium transmitting information, varies exponentially with the message length. The number of different possible messages will hereafter be called the *choice*. Thus a message choice varies exponentially with the message length.

C. Quantity of Information

Let us suppose that a message is transmitted telling about the occurrence of an event. We will define the amount of information received in the message as

$$\text{Information received} = \log \left[\frac{\begin{array}{c}\text{probability at the receiver of the}\\ \text{event after the message is received}\end{array}}{\begin{array}{c}\text{probability at the receiver of the}\\ \text{event before the message is received}\end{array}} \right] \tag{37}$$

We will begin our analysis by considering what is called the " noiseless " case. In the noiseless case the receiver is certain that the message received is correct. Therefore, the probability of the event after the message is received is unity and Eq. (37) reduces to‡

Information received (in the noiseless case)

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† These probability constraints are caused by the relative frequencies of use of the different words in the language. Probability constraints act as a means of reducing errors in the use of a language.

‡ We recall for the reader the following facts concerning logarithms :

$$g^{(\log_g f)} = f \tag{i}$$

where f and g are any numbers and $\log_g f$ is called the logarithm of f to the base g. Thus

$$2^4 = 16, \quad \text{so that} \quad \log_2 16 = 4 \tag{ii}$$

Also $\log\left(\dfrac{b}{a}\right) = \log b - \log a$ (iii), and $\log 0 = 1$ (iv)

This may also be expressed

$$\log \left[\frac{\begin{array}{c}\text{probability on the basis of knowledge available}\\ \text{at the receiver after the message is received}\end{array}}{\begin{array}{c}\text{probability on the basis of knowledge available}\\ \text{at the receiver before the message is received}\end{array}} \right]$$

$$= -\log \begin{bmatrix}\text{probability at the receiver of the event}\\ \text{before the message is received}\end{bmatrix} \qquad (38)$$

As an example, let us suppose that a baby has just been born at a neighbor's house and the question is asked, " What is the baby? " The answer, " It's a boy," then gives a specific amount of information in accordance with Eq. (38), namely,

$$\text{Quantity of information} = -\log\left(\tfrac{1}{2}\right) = \log 2 \qquad (39)$$

In the above equation, it has been assumed that the baby was equally likely to have been a boy or a girl, so that the *a priori* probability of its being a boy is 1/2.

The numerical value of the amount of information in the above equations depends upon what logarithmic base is used. If 10 is used as the base of logarithms, a unit of information may be called a decimal digit. If 2 is used as the base of logarithms, a unit of information may be called a binary digit.

It has become a universal practice to contract the term " binary digit " and use the term " bit " for the unit of information. In the opinion of the present author, this is unfortunate, since the expression " a bit of information " has a very inexact meaning in nontechnical use of the English language. Nevertheless, we shall follow the general practice and call a unit of information in the binary system a " bit ".

The use of 2 as the logarithmic base has certain advantages in information theory. We note in passing that since

$$\log_2 2 = 1 \qquad (40)$$

exactly one bit of information tells which of two equally likely possibilities has been chosen.

The following example will illustrate the significance of the foregoing definitions. Suppose that a treasure is known to be located somewhere in the area depicted by Fig. 8. Suppose the area is considered to be divided into 16 squares as shown and that a searching party is sent out to locate the treasure. Suppose the party sends back the following message to headquarters after the first day " Treasure in Row II." Assuming that the treasure was equally likely to have been in any of the rows before receipt of the message, the probability before receipt of the message that the treasure was in Row II was 1/4. Consequently, assuming that the message is completely reliable, the amount of information it gives is, by Eq. (38),

$$-\log_2(1/4) = \log_2(4) = 2 \text{ bits}$$

Next suppose that the following day the party sends back the message " Treasure in Column C." By similar reasoning, it follows that the second message also gives 2 bits of information. Thus, by sending back 4 bits of information, the searching party has informed headquarters of the exact square of location of the treasure.

Instead of sending two messages in the foregoing manner, suppose that the searching party had only sent a single message, "Treasure in 10th square." It is simple to show that the same amount of information has been required to locate the treasure as in the preceding case. Thus, before the message is received, the probability that the treasure was in the 10th square was 1/16. The amount of information in the message is therefore

	A	B	C	D
I	1	5	9	13
II	2	6	10	14
III	3	7	11	15
IV	4	8	12	16

FIG. 8. Mapping an area.

$$-\log_2 (1/16) = \log_2 (16) = 4 \text{ bits}$$

If our answer in the second case had been different from that in the first, then Eq. (38) would thereby have been shown to lead to inconsistencies and would have had to be abandoned.

In the first case, in which two messages were sent, it was proper to add the entire information of the two messages, since the information given by each message was independent of that given by the other. In cases where there is redundancy of some of the information in messages, it is not possible simply to add the information given by separate messages. In such cases, more involved methods are necessary to determine the total quantity of information.

D. Information and Choice

An elementary system for the transmission of information deals with only one topic. For example, suppose we have an unattended weather station (Fig. 9) where readings of temperature, barometric pressure, and relative humidity are automatically encoded as a single message and transmitted by radiotelegraphy to a weather study center 200 miles

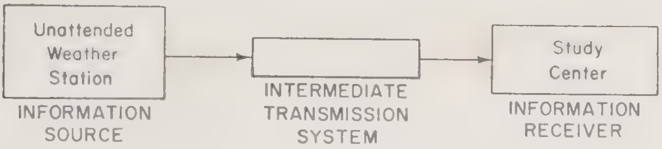

FIG. 9. An elementary information transmission system.

away. This is an elementary type of information transmission system, since each message deals with only one topic, namely the weather condition at the station. The special characteristic of an *elementary* information transmission system is that the fraction of all the messages transmitted in the system which tell that a particular event, namely a particular weather condition in the foregoing example, has occurred is exactly equal to the probability of the occurrence of that event.

Let us suppose that there are r different weather conditions which can be recorded at the weather station. Let us number these conditions 1, 2, 3, . . . up to r. Then let p_1 be the probability of occurrence of condition 1, p_2 be the probability of the occurrence of condition 2, and so on. Then if we introduce a new symbol h defined by the equation

$$h = -[p_1 \log p_1 + p_2 \log p_2 + \dots + p_r \log p_r] \tag{41}$$

it may be shown that h is equal to the average amount of information per message sent from the weather station. The same statement holds for any elementary information transmission system. The quantity h is usually called the *entropy per message* of the information transmission system. It may further be shown that the total choice in a long sequence of m messages—if the sequence is required to have a fractional distribution equal to the probability distribution of the message—is

$$\text{Total choice} = A\ 2^{hm} = (L')^n \tag{42}$$

In Eq. (42) it is assumed that each message is expressed as a group of symbols and n is the total number of symbols in the sequence of messages. From Eq. (42) it follows that

$$\log_2 [\text{total choice}] = hm + \log_2 A \tag{43}$$

If m is a large number, it follows that $\log_2 A$ is negligible, so that

$$\log [\text{total choice}] = hm = \begin{bmatrix}\text{total amount of information} \\ \text{in the sequence}\end{bmatrix} \tag{44}$$

Eq. (44) gives the basic relation between information and choice. Actually if the probabilities of the different events are equal, then Eq.

(44) holds even if m is not large. For example, in the case discussed earlier where there were two equally probable events :

1. It's a boy,
   and
2. It's a girl,

the total choice for one message ($m = 1$) was 2, and the information per message was one bit [Eqs. (39) and (40)].

In closing this subsection we wish to emphasize once more the basic importance in information theory of the exponential law of choice as illustrated in Eqs. (36) and (42). Coupled with the logarithm of choice (or probability) as a quantitative measure of information, this law allows information rates to be assigned to information transmission systems and information sources.

E. Information in Continuous Curves

Figure 10 shows a diagram of a continuous recording of a quantity whose value varies with time, for example, a temperature or a chemical concentration. It is of interest to know how such a diagram is related to the sequences of symbols described earlier in this section.

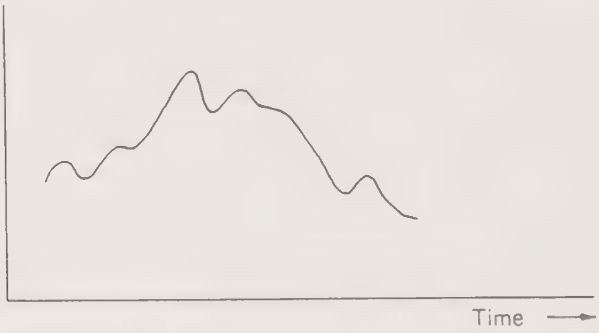

FIG. 10. A continuous signal. The ordinates may be a quantity such as temperature or concentration.

It can be shown (Goldman, 1953, Chapter 2) that a continuous signal such as shown in Fig. 10 is in effect a sequence of symbols. This demonstration is accomplished by showing that the maximum rate at which a variable can change back and forth with time in any actual physical system has an upper limit. This limits what is called the *frequency bandwidth*† of the signal in Fig. 10. It is then shown that if one knows the values of a signal of maximum bandwidth W at times $1/2W$ apart, one has sufficient information to fill in the curve between these specified times. These specified times $1/2W$ apart are called sample points of the curve. The statement above thus tells us that if we know the values of a variable at its sample points, its values at all intermediate times are completely determined. From an information point of view, a continuous curve is then completely equivalent to a sequence of values at the sample points.

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† The bandwidth of any particular system can be determined from its elementary physical properties, e.g., the configuration of inductance, capacitance, and resistance elements in the case of an electric network.

Let us next consider the possible values at any particular sample point. Suppose that the curve in Fig. 10 shows electrical potential *versus* time. In all actual cases there is a limit to the accuracy of the recording system. Some of the inaccuracy can be eliminated, but, according to information theory, part of the inaccuracy is theoretically irreducible. It may be shown that this reduces the possible values of voltage to a finite number of different effective subdivisions in any range. Thus, it may be that if the potential (in volts) is specified to the fourth decimal place, any attempt at greater accuracy is meaningless and carries no information. In any physical system there will of course be an upper limit to the largest possible value of the recorded signal. The net result is that the different possible values of significance form an alphabet of signals of finite size, say L. From the point of view of information theory, the curve in Fig. 10 thus represents a sequence of n sample point values, each drawn from an alphabet of L possible values. The representation of a continuous curve by such a sequence is called a *quantized representation* of a curve.

F. Errors and Redundancy

1. *Predictable Errors*

If a signal is sent from point A to point B (Fig. 11), there may be errors in the signal arriving at B, even though these were not present at A. These errors can be divided into two classes, predictable and unpredictable. The words " predictable " and " unpredictable " are used here in a technical sense. If a given transmitted signal at A will always

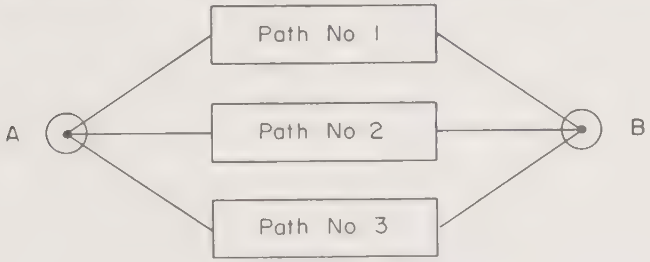

FIG. 11. Redundant transmission paths.

give rise to the same received signal at B, then any error in the received signal is said to be predictable. In the predictable case, if the received signals are different whenever the transmitted signals are different, in other words, if there is a one-to-one correspondence between transmitted and received signals, then any errors are equivalent to errors in translation and can be eliminated by correction of the dictionary of translation. On the other hand, if in the predictable case, several different transmitted signals will give rise to the same received signal, then there is ambiguity in reception, since an observer at the receiver does not know precisely which signal was transmitted. This ambiguity causes a decrease in the received information and is considered a predictable error.

2. *Unpredictable Errors*

If a given transmitted signal at A will not always give rise to the same received signal at B, then there are unpredictable errors in the received signal. In information theory, unpredictable errors are called *noise*. Both in technical systems and the natural world, unpredictable errors in reception are reduced by *redundancy*. In its simplest form, this is repetition of the signal. The signal may be repeated in time, as is frequently done in conversation, or the redundancy may be accomplished by sending the same signal by way of several independent paths between A and B (see Fig. 11). There are also more complex and sophisticated forms of reduncancy. A relatively simple physiological use of redundancy is described in Section IV, C.

G. Some Matters of Translation and Transmission

When information is translated from one language to another, an equivalence is set up between messages in the message alphabet of one language and those of the other. If each alphabet consists of a discrete set of messages, the equivalence can readily be set up, even though the messages are of different form. Thus XVIII may be translated into 18. When information is transmitted in the form of continuous variation (equivalent to continuous curves), it is extremely difficult to translate the information into another language, unless the latter is of basically the same form as the first. However, if a mechanism is available to translate a continuous curve into a quantized representation, further translation into other languages then becomes a much simpler matter.

Also of considerable interest is the difference between quantitative and qualitative information with regard to transmission errors. If quantitative information—which conveys magnitude—is expressed as the ordinate of a signal which becomes attenuated in transmission, then the information is changed because there is a loss of signal. On the other hand, qualitative information is less susceptible to errors caused by attenuation. Both a large and a small apple are still apples. Qualitative information is generally less susceptible to the various possible types of error which can occur in the transmission of information. Qualitative information is also very versatile in translation. For these reasons, it is sometimes desirable to translate quantitative into qualitative information before transmission.

H. Concluding Remarks

It may be useful to conclude by listing some important facts of information theory to see what light they can throw on homeostasis.

1. Information is the equivalent of the characteristics of a situation (event or idea) translated into a form suitable for communication.
2. An information transmission system has a list of possible messages to each of which a meaning is assigned. (A signal to which no meaning is assigned is not considered a message.)
3. Messages can be translated from one language (or code) into another without changing their information content.
4. Nonpredictable (random) errors in the transmission of information may be reduced by redundancy, which in its simplest form is the repetition of messages in one way or another.
5. The wave shapes of continuous curves have equivalent quantized representations for information transmission purposes. These quantized equivalent signals are simpler and more versatile in translation.
6. The exponential law of choice, the relation between probability and choice in long sequences, and the use of the logarithm of probability as the measure of information, form the mathematical basis for the theory of information.

In our discussion of the application of information theory to homeostasis in the next section, we shall find items 1 through 5 quite pertinent. It appears, however, that item 6 is too sophisticated to be of use in the present state of knowledge of the experimental facts of homeostasis.

IV. Applications to Biology, Especially to Homeostasis

A. The Control of Blood Glucose†

We shall now endeavor to apply the ideas and thinking of control theory and information theory to homeostasis. Because of the variety of topics which it will illustrate, we shall begin our discussion by considering certain aspects of the control of blood glucose. In Fig. 12 is a diagram showing parts of the blood glucose control system of the body in a form instructive for our purposes. This is not intended as a complete picture of the blood glucose control system, but shows only those

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† The control of blood glucose, rather than some topic in mineral metabolism, is used in this chapter as the principal example to illustrate the application of cybernetic theory because the author does not know of any topic in mineral metabolism about which there is sufficient experimental information to give such a wide range of illustrative material.

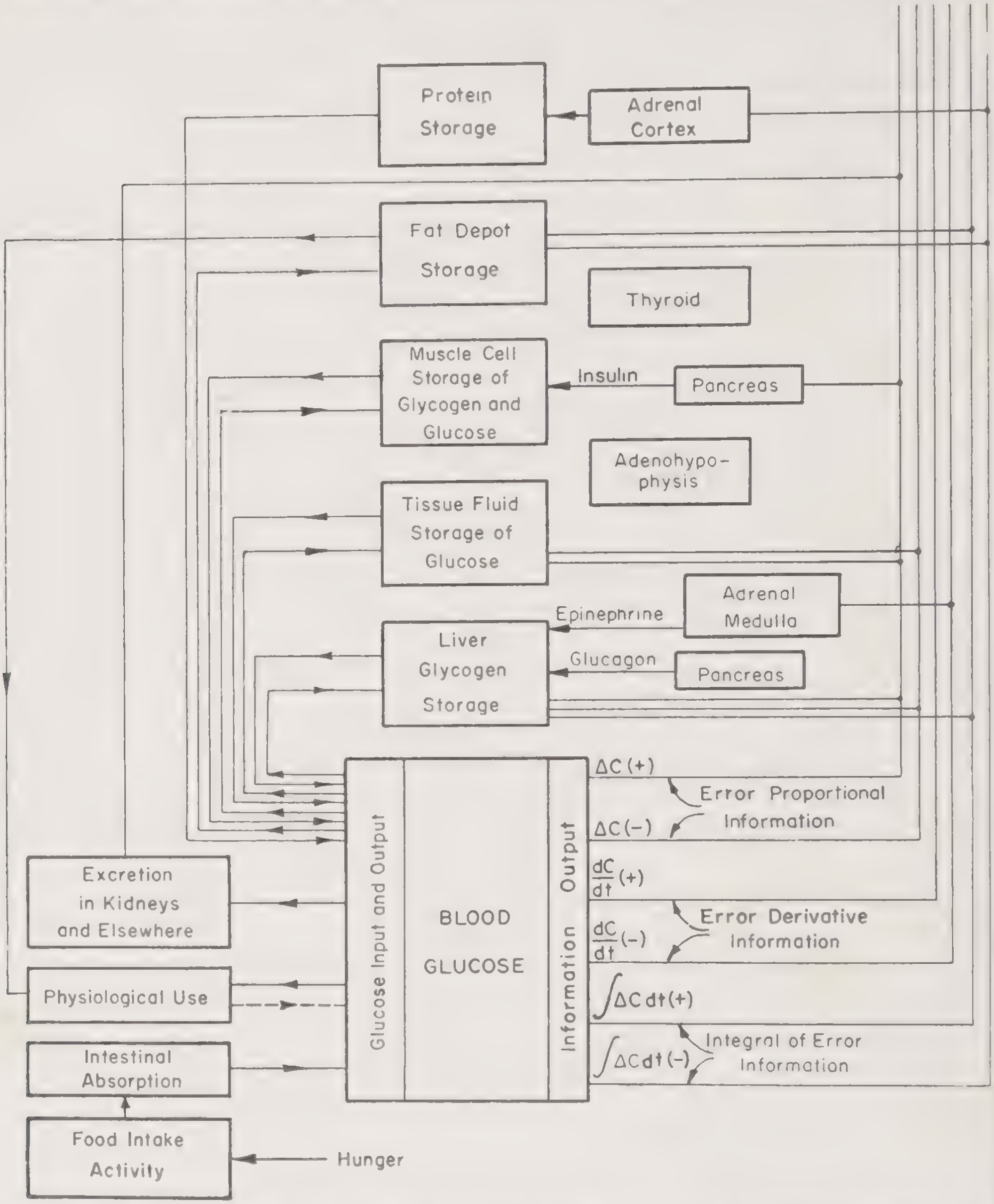
Protein Storage
Adrenal Cortex
Fat Depot Storage
Thyroid
Muscle Cell Storage of Glycogen and Glucose
Insulin
Pancreas
Adenohypophysis
Tissue Fluid Storage of Glucose
Adrenal Medulla
Epinephrine
Liver Glycogen Storage
Glucagon
Pancreas
ΔC (+)
Error Proportional Information
ΔC (−)
dC/dt (+)
Error Derivative Information
dC/dt (−)
∫ΔC dt (+)
Integral of Error Information
∫ΔC dt (−)
Glucose Input and Output
BLOOD GLUCOSE
Information Output
Excretion in Kidneys and Elsewhere
Physiological Use
Intestinal Absorption
Food Intake Activity
Hunger

FIG. 12. CERTAIN ASPECTS OF THE BLOOD GLUCOSE CONTROL SYSTEM. Only those pathways discussed in the text are shown. Others certainly exist. The pathways indicated by the arrows may be via the blood circulation or via the nervous system or both. Pathways may also go via other means.

aspects which illustrate the application of the ideas developed in the earlier sections of this chapter.

At the right of the BLOOD GLUCOSE compartment are six emerging information lines, indicating the types of information concerning blood glucose which the discussion in Section II would lead us to expect to be of interest. Let us say that the desired (quiescent) value of blood glucose concentration is about 100mg%. When the actual value in the blood glucose compartment is above the quiescent value, this information may be considered as going out on the $\Delta C(+)$ line to those parts of the regulatory system which react to a higher than quiescent value of blood glucose. In some cases an indication may also be given of the amount by which the blood glucose value is too high. When the value of blood glucose concentration is low, the analogous information goes out on the $\Delta C(-)$ line. The $\Delta C(-)$ line is distinguished from the $\Delta C(+)$ line because its information does not necessarily go to the same set of organs.

Next consider the error derivative information dC/dt. When the concentration is falling (dC/dt is negative), this information is transmitted to the adrenal medulla, which then secretes epinephrine that is carried to the liver ; the liver in turn converts stored glycogen to glucose, which is released to the blood. It is possible that $(dC/dt)(+)$ information goes to the pancreas to stimulate the release of insulin, but whether $\Delta C(+)$ or $(dC/dt)(+)$ information or both stimulate the release of insulin is not clear from the literature.

Integral-of-error information $\int \Delta C dt(+)$ and $\int \Delta C dt(-)$ both apparently get to the fat depot organ, the former possibly stimulating lipogenesis and the latter stimulating fat mobilization. Negative integral-of-error information gets to the protein storage of the body by way of the adrenocortical hormones to stimulate gluconeogenesis.

The foregoing paragraphs in conjunction with Fig. 12 have indicated some of the pathways by which information on the blood level of glucose gets to the body reservoirs and other regulatory devices which keep the blood glucose concentration under control. Of special interest is the fact that all six types of information indicated in the figure appear to be used in the regulation of blood glucose ; to some extent, at least, the body distinguishes between them and makes different use of them.

There is evidence to indicate that the liver glycogen is acted upon in different ways by proportional, derivative, and integral controls. If the liver is perfused by blood relatively free from epinephrine, it appears to be stimulated to store glucose as glycogen if the blood glucose level is high and to release glucose from glycogen storage if the blood glucose is low. This is a type of proportional control. On the other hand, the release of glucose from stored glycogen, as induced by epinephrine, seems to be a derivative control, i.e., a response to $(dC/dt)(-)$. Finally, lipogenesis in the liver appears to be an integral control.

In the foregoing paragraphs, we have classified certain known controls of the body glucose as proportional controls, others as derivative controls, and still others as integral controls. Although the statements made seem reasonable, we actually have relatively little quantitative information in the literature upon which to base them. When experiments are made on a regulatory system, it would be desirable from the point of view of servo theory to determine whether a control is of the proportional, derivative, or integral type. For example, it would be of interest to know how the pancreas responds to the height of blood glucose, to the rate of glucose rise, and to the time integral of excessive glucose concentration. The same information would also be of interest about the adenohypophysis and other regulators. The present author is not competent to judge how much of this information is already in the literature. It would appear, however, that a proper servo classification of the available details of the control system would help in revealing the nature of the chemical and biological mechanisms involved. Although it may be very difficult to obtain the data required for the above-mentioned classification, it may be helpful to keep this classification in mind as an objective.

B. Antagonists and Instability

The discussion in Section II indicated that for efficient control, any controlling agent which operates only in one direction should have an antagonist available. In the glucose regulatory system, one unidirectional controlling agent is insulin. Insulin lowers the blood glucose. A hormone of the adenohypophysis, in conjunction with an adrenocortical hormone, operates as a general antagonist for insulin and has the effect of raising the blood glucose. The adrenocortical hormone apparently promotes gluconeogenesis, thus making more glucose available. The mechanism of action of the adenohypophysal hormone is not certain, but one knows that it tends to cancel the effect of insulin. It is not known whether this adenohypophysal hormone is secreted in response to a low blood glucose, to a falling blood glucose, or in response to some other cause. Furthermore, it is generally believed that another hormone, glucagon, originating in the pancreas, but in cells different from those secreting insulin, is also important in the control of blood glucose. Glucagon stimulates the conversion of stored liver glycogen into glucose for release into the blood. Part of the action of the adenohypophyseal hormone may be that it stimulates the release of glucagon. The foregoing discussion indicates that while physiological antagonisms exist in the control systems of the body, the antagonists are not necessarily arranged in pairs having opposite functions.

The discussion in Section II, C indicates that when antagonistic activities are present, there is a danger of instability (" vicious cycles "). One may therefore consider whether particular symptoms or diseases are due to a servo type of instability. In order to estimate whether this is likely, it is very desirable to know quantitatively the separate time-course of action of the controlling agent and its antagonist as well as the magnitude of the feedback factors β (Section II, C). Such data are at present unavailable for any biological system.

In this connection it is of interest to consider whether diabetes is a result of servo instability. A diabetic individual has insufficient insulin in his blood stream to meet his requirements. If the cause of the insufficiency is not the result of erroneous control activity, servo instability is not involved. If, on the other hand, there is overactivity of the insulin antagonists and the pancreas is unable to supply sufficient insulin to balance this overactivity or wears out or degenerates in the attempt, then servo instability is certainly involved. The case would most clearly be a classic type of servo instability if the trouble were due to an error in timing by either insulin or its antagonist so that either too much or too little cancellation took place. Thus if the antagonist acted too soon, excessive insulin would be required. On the other hand, if the action of the antagonist were late, the insulin would have too much effect. This in turn might call forth excessive antagonist activity and thus call forth more insulin and in this way develop into a vicious cycle. The foregoing discussion of diabetes may not do full justice to our knowledge of diabetes, but it calls attention to what has possibly not been appreciated : namely, that improper timing of antagonistic activity can lead to severe trouble in a body-regulating system.

In looking for an example of a case which might possibly depict instability in homeostatic regulation of electrolytes, it seems worthwhile to consider certain types of edema. Here, excessive retention of sodium is accompanied by excessive water retention. Greater than normal amounts of aldosterone (the salt-retaining hormone) have been observed in the urine and also in the blood of patients with edema. The overproduction of aldosterone is, however, not the original malfunction involved. It would therefore appear that this overproduction is an error of the regulatory system, inasmuch as reduction of aldosterone would seem desirable.

There is evidence to indicate that the secretion of aldosterone increases in response to a fall in the effective circulating volume of blood. In a healthy man, a decline in the effective circulating volume of blood involves a loss of sodium from the body. An increased level of aldosterone then causes the retention of sodium by the kidneys. This increases body sodium, which in turn tends to increase blood fluid volume and ultimately the effective circulating volume of blood.

A possible explanation of the excess of aldosterone associated with edema would be as follows. First there is a fall in the effective circulating volume of blood, without a loss of sodium from the body. For example, in cardiac edema the loss in effective circulating volume may first be due to a weakness of the heart pump. In nephrotic edema, the loss in circulating volume may be due to renal loss of blood protein. This loss of protein causes a transfer of fluid from the blood to the tissues. In either of these cases, the regulatory system then responds by increasing the level of aldosterone, which increases the total body sodium above the normal level. Most of this excess sodium does not remain in the blood, but enters the tissue fluid and in turn causes an increased retention of water in the tissues. The original cause of the low circulating volume has, however, not been corrected and any rise in the effective circulating volume obtained by the abnormally high level of total body sodium still does not bring it up to the desired level. Furthermore, the accumulated tissue fluid probably impedes the circulation of blood. The regulatory system therefore continues to supply excessive aldosterone, increasing blood sodium, tissue fluid sodium, and ultimately tissue fluid volume until marked edema has developed. Whether or not the foregoing is the true explanation of various edemas, it illustrates the possibility of a disease of this type being due to a vicious cycle type of servo instability (cf. Vol. I, Chapter 10 and Vol. II, Chapter 25).

C. Applications of the Ideas of Information Theory

In Subsections A and B, we discussed principally application of the ideas of servo theory. We now wish to consider how the ideas of information theory can help in dealing with homeostasis. At the outset it should be stated that little has been done along this line. As workers in the field of homeostasis become better acquainted with information theory, one can expect more profound and more subtle applications than those presented here.

One aspect of information theory which can be recognized in many biological phenomena is the use of redundancy for error reduction. Thus in Fig. 12 we see five different mechanisms for the storage of glucose or its equivalent. Even though each of these has a different specific function, if any one of them gets out of order, the others will work in the direction of correcting the error caused by it. The type of redundancy displayed here, where each redundant element has a different method of operation, is a very effective protection against major errors, since it is unlikely that many of the redundant elements will get out of order at the same time or due to the same cause. This type of combination of qualitative and quantitative redundancy represents a safety factor against disease or malfunction.

Another aspect of biological phenomena of interest from the point of view of information theory is the widespread use of multidimensional† signals, often quantized. Thus the state of the blood glucose is apparently transmitted to the regulatory systems of the body in terms of the states of the six variables indicated as information output lines in Fig. 12. Some of these lines (e.g., to the adrenal medulla), especially when they go to an organ of secretion via the nervous system, may only carry the binary information that the signal is either present or absent. The information going to the tissue fluid storage merely operates on the basis of diffusion and other physicochemical phenomena and is almost certainly not quantized. On the other hand, the information going to the fat depot storage and protein storage is probably not binary, but may be quantized.

Binary information is of the nature of qualitative information in that it is unaffected by attenuation. It is thus immune to errors caused by fluctuations in magnitude. It is also basically very simple. These characteristics make binary information desirable for use in the body regulatory systems. In fact, it would be of interest to determine to what extent homeostatic regulation is based only upon such binary signals used in conjunction with organ adaptation.

There is one question which is certainly at the center of any adequate treatment of the regulatory systems of the body from the point of view of cybernetics. This is the question of what determines the quiescent values, such as 140 meq/liter sodium or 100mg% glucose in the blood. Much of the regulatory information sent around the body involves these quiescent values, and any proper elucidation of the control system must tell what determines these values. Cybernetics does not answer this question, but emphasizes its importance.

D. Remarks Concerning Experimental Problems in the Study of Physiological Control Systems

Let us examine briefly the problems involved in ascertaining experimentally the magnitude of a given control and of determining whether it is a proportional, derivative, or integral control. As an example, let us once more consider the control of blood glucose. Our original discussion of control systems in Section II was idealized and simplified in many ways. We must now remove some of these idealizations in order to fit an actual physiological situation. To some extent this has already been done but we now wish to deal with the problem directly.

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† A multidimensional signal is one which is expressed in terms of several variables.

In our earlier idealized formulation of the problem, which is shortly to be modified, it was assumed that blood glucose has a desired quiescent concentration of some specified value, say 100 mg%. This desired value remains fixed. If the concentration rises, one or more proportional controls come into play to bring the concentration back to the desired value. Furthermore the magnitude of the action of the proportional control is proportional to the magnitude of the deviation of the concentration from the desired value. In addition to the proportional controls, there are also derivative controls. These have an action whose magnitude is proportional to the rate of change of the concentration and which act to oppose the change. Finally, there are integral controls. The magnitude of these is proportional to the time integral of the deviation from the desired concentration, and their effect is to bring the concentration back to the desired value.

The actual physiological situation differs from its idealized counterpart in many ways which tend to confuse the experimental picture. In the first place, the quiescent level is not constant, even for a given individual. It varies with time, perhaps as an adaptation phenomenon to changing physiological conditions. In experimental observations, this variation of the quiescent value makes it difficult to determine the magnitude of the deviation of the actual concentration from the quiescent value.

Consider next the action of the proportional controls. In the idealized case, they have a magnitude of action which is proportional to the deviation of the actual concentration from the quiescent value. In fact the name " proportional control " is sometimes taken to imply that the action is proportional to the " error " in concentration. We, however, use the term to mean that the *information to which a proportional control responds* is proportional to the *magnitude* of the error. The magnitude of the action of a proportional control need not be proportional to the magnitude of the error. The curves in Fig. 13 illustrate some of the possible ways in which the action of a proportional control may vary with the concentration. In the case of Curve (1), the control action is proportional to the magnitude of the error. In Curves (2), (3), (4), and (5) it is not. Curve (4) illustrates a quantized type of control having a number of thresholds. Curve (5) illustrates a type of control which is inactive over a wide range of concentrations but becomes very active outside this range. The loss of glucose in the urine is of the latter type.

Let us next consider derivative controls. In the idealized case, the action of a derivative control is proportional to the time rate of change of the concentration as in Fig. 14 Curve (1). In the physiological case, however, the regulatory action of a derivative control needs only to respond to the rate of change of concentration ; it does not have to be proportional to it. Furthermore, it may also depend upon other parameters, such as the actual value of the concentration as well as upon its rate of change. Thus, if the action of the adrenal medulla in sending epinephrine to the liver is indeed a derivative control, the magnitude of this action needs only to depend on the rate of fall of glucose concentration : it does not have to be proportional to it. Furthermore, for the same rate of fall of glucose concentration, the magnitude of response of this mechanism may well be different for different levels of glucose concentration. Thus while Curve (2) in Fig. 14 may be the correct description of how a derivative control would act in a certain physiological condition, it would be very difficult to determine this curve experimentally because parameters other than the rate of change of concentration may vary during the experiment and have the effect of changing the applicable curve from Curve (2) to, for example, Curve (3) in the course of the experiment.

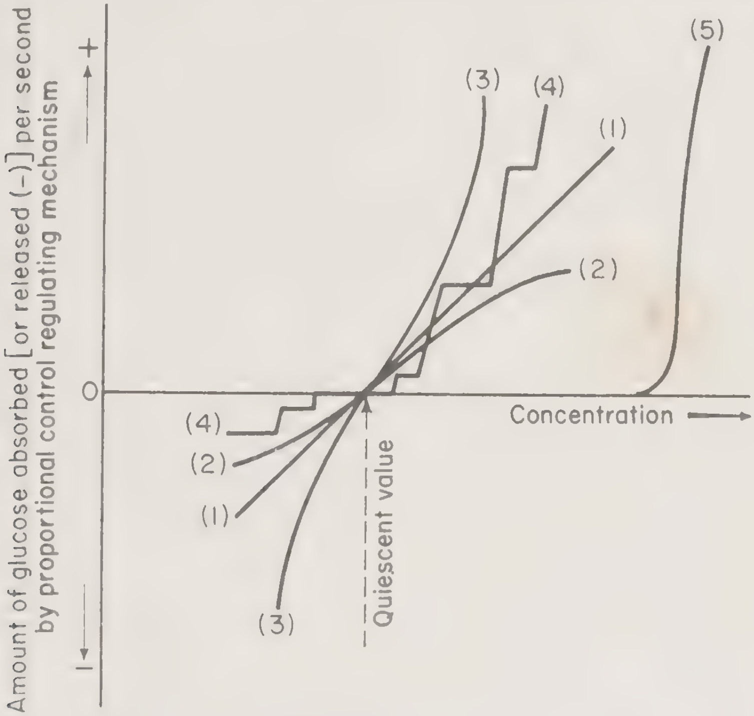

Fig. 13. Various types of proportional control.

A similar possibility of variability exists in the physiological action of the integral controls. Their action will respond to the time integral of the concentration error, but need not be proportional to it. The magnitude of the integral control action may also be affected by a number of other physiological parameters.

The foregoing paragraphs have pointed out some of the difficulties which may be encountered experimentally in trying to isolate and measure the effect of various proportional, derivative, and integral controls. There are still other difficulties. Thus the control characteristics, as shown by the curves in Fig. 13 and 14, may change with time as an adaptation to the life history of the animal under test. This description as an adaptation may actually just describe the effects of all those extraneous parameters which affect the experimental measurements, but which are difficult to specify and hold constant. Other related difficulties may be described as fatigue and exhaustion phenomena in physiological regulatory systems. Finally, it will be obvious that the study of any one regulatory mechanism will be plagued by the simultaneous operation of all the other regulatory mechanisms which are not specifically under study.

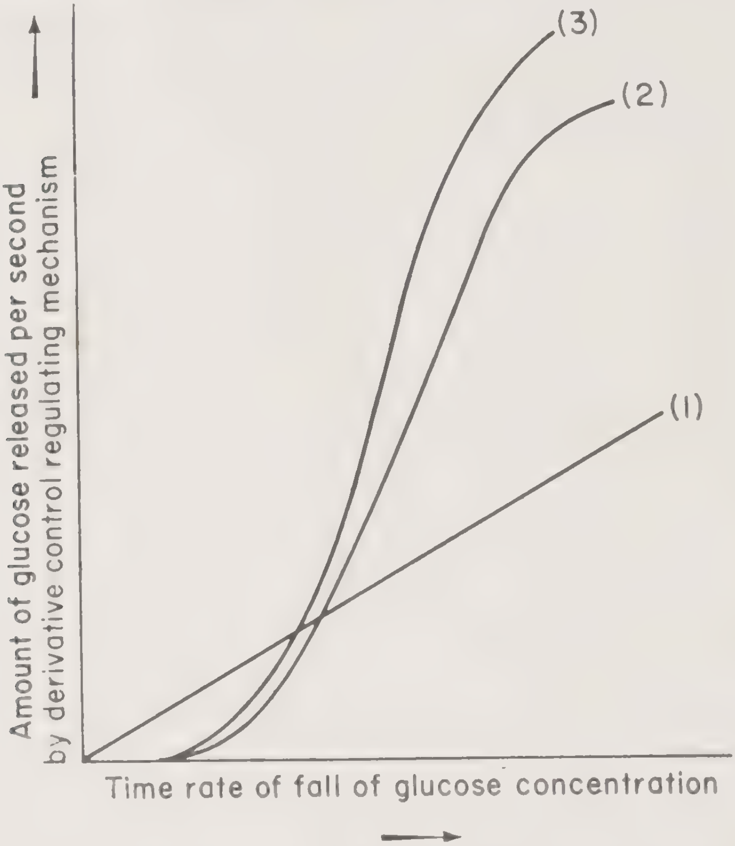

Fig. 14. Types of action of derivative control.

In the face of such a formidable array of possible difficulties, and in order to obtain pertinent data on the operation of the regulatory mechanisms, it is necessary to tailor one's experiments to the particular job at hand. For example suppose that a careful study of available knowledge indicates that when important changes in concentration take place in a few minutes or less, then the actions of the derivative controls predominate and those of the other controls may be neglected. With the aid of this knowledge a large number of difficulties may be removed in setting up experiments to study derivative controls. In other cases, it is sometimes possible to study a control system or part of it as an isolated entity. It is beyond the capability of the author to outline definite procedures for general use in obtaining the desired experimental data which describe the action of biological regulatory systems. It is, however, reasonable to say that an important result of the study of a regulatory system would be a curve of one of the types shown in Figs. 13 or 14, as well as (*a*) a specification of the conditions under which the curve can be obtained and (*b*) a specification of the conditions under which the curve will describe the action of the regulatory system. These curves, besides being a quantitative description of the action of the regulatory system, should act as a test for existing theories of the detailed biochemical and/or biophysical mechanism and they should also be helpful in suggesting new theories.

E. The Control of Biological Characteristics

The characteristics of an organism are determined by the genes in its chromosomes. It is therefore an intriguing task to apply information theory to the transmission of this information from the genes to the rest of the organism. Along this line, Goldman (1948) suggested that the presence of a replica of the genetic information in the chromosomes of every cell in the organism may be a " noise reduction " mechanism which tends to improve the correspondence between the organism and the specifications called for by the genetic information. Gamow and associates (1956) have applied certain aspects of coding theory to the information transfer from nucleic acids to proteins. In a very thoughtful book, Quastler (1953) and others have examined the possibility of applying information theory to various biological phenomena.

The template theory, which relates nucleic acids to the synthesis of proteins, and the related template theories in the studies of antimetabolites and immunology deal with mechanisms for the translation of information. The above-cited work of Gamow and his co-workers is probably the most direct application of methods related to information theory along this line. These fields are very active and important and may witness important applications of information theory in the next few years.

The existing evidence indicates that genes are large molecules. According to modern physical theory, they should therefore, at any time, be in particular quantum states. While we do not know how the information in the genes is translated into characteristics of the organism, one would be inclined to expect that the information in a gene consisting of a molecule in a particular quantum state would be translated into strictly specified characteristics. A change in a quantum-molecular state of a gene would give rise to a definite mutation rather than to a small variation in a given characteristic. As pointed out by Schrodinger (1945), this point of view is in apparent agreement with the evidence of genetics. This point of view is also in agreement with the evidence of taxonomy, since the plant and animal worlds consist of distinct species, and a continuous range of individuals with intermediate characteristics does not exist. According to this point of view, the difference in a characteristic of two individuals, where this characteristic is determined by *identical* genes, is not caused by a difference in the genetic material, but by random environmental fluctuations involved in the control systems which translate the gene information into the characteristics of the organism.

A critical part of the translation of gene information into the characteristics of the organism occurs during embryological development. A beginning in the experimental elucidation of what occurs during this period has been made (see Waddington, 1956), but not yet at the level of the detailed mechanism or control system. In the adult individual, the genetic information has apparently been translated into a stable organism with a set of fully developed regulatory systems. These will maintain the characteristics of the individual, despite variations in the external environment and despite fluctuations in the relation of the individual to the environment. The fact that genetic information can be translated into characteristics with a recognizable permanence existing for years is due in large part to the effectiveness and stability of these regulatory systems.

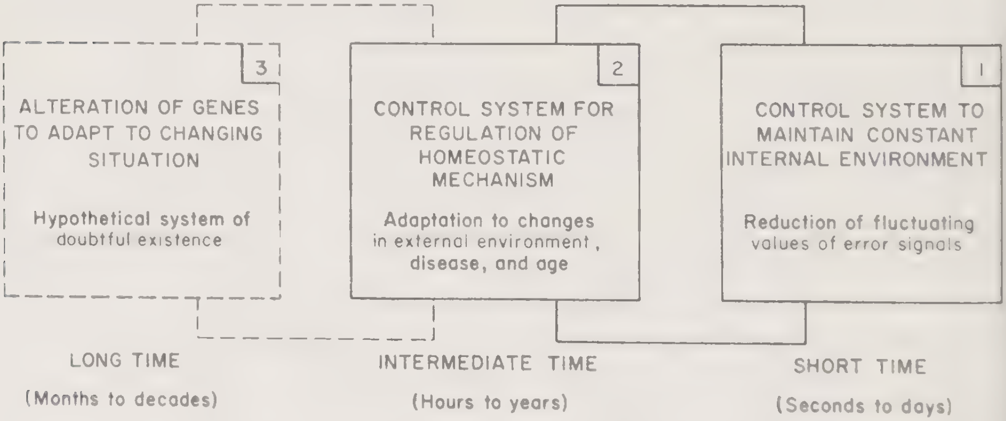
3
ALTERATION OF GENES TO ADAPT TO CHANGING SITUATION
Hypothetical system of doubtful existence
2
CONTROL SYSTEM FOR REGULATION OF HOMEOSTATIC MECHANISM
Adaptation to changes in external environment, disease, and age
1
CONTROL SYSTEM TO MAINTAIN CONSTANT INTERNAL ENVIRONMENT
Reduction of fluctuating values of error signals
LONG TIME
(Months to decades)
INTERMEDIATE TIME
(Hours to years)
SHORT TIME
(Seconds to days)

FIG. 15. Cascaded stages of biological control.

The regulatory systems of an individual may be looked upon as an adaptation system. This is depicted in Fig. 15. Compartment 1 on the right represents a control system which deals directly with error signals. The blood glucose control system in Fig. 12 is such a system. It adapts the organism to deal with the environment on a short-time basis. These times are of the order of magnitude of seconds, minutes, or hours, or at the most, days. Compartment 2 is a regulatory system which changes the characteristics of the organs and mechanisms in the control system of Compartment 1 in order to adapt them to new conditions, as the average environmental conditions change with time. The times involved in the regulation performed by Compartment 2 are of the order of magnitude ranging from hours to years. Compartment 2 is involved in adaptating the individual to average changes in external environmental conditions and to such internal changes as aging and disease.

Compartments 1 and 2 represent control systems which certainly exist, even though we do not know how they work. Compartment 3 represents a hypothetical control system which may not exist at all. However, its existence is somehow suggested by Compartments 1 and 2. Compartment 3 represents a control system which regulates the characteristics of the genes to adapt them to the conditions met in Compartment 2, just as the elements in Compartment 2 are regulated to adapt them to conditions met in Compartment 1. If Compartment 3 really exists, and if the alterations in gene characteristics which it brings about occur also in the germ cells, then we would have an inheritance of acquired characteristics. When we know more about the mechanisms at work in Compartment 2, we will be better able to judge whether there is enough likelihood of the existence of Compartment 3 to justify looking for direct evidence to confirm it.

F. Concluding Remarks

The reader may well be disappointed by the foregoing account of the cybernetic aspects of homeostasis because cybernetics cannot claim any major accomplishments in the field of homeostasis.† While this is true, it is perhaps too early to expect cybernetics to be responsible for important advances in the field. It is fair to say that cybernetics has already influenced thinking about homeostasis and that it has helped in the understanding of some physiological regulatory mechanisms. It cannot replace the experiments on which biological knowledge is based, but it can help in their planning and interpretation.

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† In the past, the ideas of cybernetics have been more widely used in studies of neurophysiology than in homeostasis.

ACKNOWLEDGMENTS

The writer is indebted to Dr. Jay Tepperman and Dr. Walter Baum for valuable discussions during the preparation of this chapter.

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CHAPTER 4

Chelation

THERALD MOELLER AND E. PHILIP HORWITZ

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I. Introduction

Although A. Werner and other early workers in the field described many coordination compounds† in which a single ligand simultaneously satisfied two coordination positions, the term *chelate* was first applied to such substances by Morgan and Drew (1920), who stated " the adjective ' chelate ', derived from the great claw or ' chela ' (' chely ') of the lobster or other crustaceans, is suggested for the caliper-like groups which function as two associating units and fasten to the central metal atom so as to produce heterocyclic rings."‡ By common consent, hydrogen may substitute for a metal in such a heterocyclic ring. Compounds containing chelate rings are often called *chelates* in current terminology. *Chelation* is the general phenomenon of formation of heterocyclic rings of the type described. Ligands, the structures of which permit simultaneous attachment to a cation at two points, are termed *bidentate*. Similarly, *tri-*, *quadri-*, *polydentate* ligands can be described. Early information on chelate rings is summarized by Diehl (1937). Comprehensive discussions are given by Hein (1950), Martell and Calvin (1952a), and Parry (1956a).

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† A coordination compound is a compound of new and differing properties that is formed by the union of two or more independently existing molecular or ionic species.

‡ A case in point is ethylenediamine, $H_2NCH_2CH_2NH_2$, which can simultaneously bond to a cation through both nitrogen atoms.

A. Chelate Compounds vs. Non-chelate Compounds

Because the metal ion in any complex species is part of a new structure and is in a different chemical environment, its properties differ markedly from those it exhibited in the uncomplexed (commonly hydrated) form. This is particularly true in chelate species because of the more complete shielding of the metal ion from external influences and its participation in bonds that are commonly highly covalent. The effect is further enhanced when the metal is at the same time a component of more than one ring. Chelate compounds are commonly highly stable with respect to dissociation into their components and have properties similar to those of covalent organic species. These are exemplified both in naturally occurring chelate compounds (e.g., chlorophyll, hemoglobin, vitamin B_{12}) and those synthetically prepared (e.g., derivatives of 1,3-diketones, amino acids, 8-quinolinols). By the same token, the presence of a metal ion in a chelated structure alters both the chemical and physical characteristics of the organic ligands.

B. Methods of Establishing Chelate Ring Formation

These methods are commonly the same as those employed to establish the formation of non-chelate complex species, but proper interpretation of results often permits conclusions as to the presence or absence of chelate rings. Among the more common of these methods are those listed below. (See also Martell and Calvin, 1952, pp. 19–75.)

1. *Chemical Methods*

Chemical methods are: isolation and characterization of pure crystalline compounds; comparison of reaction rates in the presence and absence of the suspected chelating agent; evaluation of exchange reactions involving this agent; resolution of compounds where composition suggests optical isomerism.

2. *Physical Methods*

Physical methods are: alteration in properties of metal ion or chelating agent in terms of changes in absorption spectrum, conductance, ion migration, oxidation potential, pH, magnetic moment, or solubility; determination of crystal structure by X-ray methods.

II. Nature and Structure of Chelate Rings

A. Groups Involved in Chelate Rings

Donor atoms, i.e., atoms bonded to cations in chelate species, are most commonly oxygen, nitrogen, and sulfur. These are relatively small and normally quite highly electronegative atoms. Occasionally, they appear in purely inorganic donors such as carbonate or sulfite ion.

More commonly, however, they are found in organic structures. A few representative donor groups are summarized in Table I, where

TABLE I

TYPICAL CHELATING GROUPS

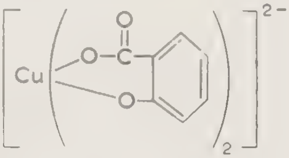
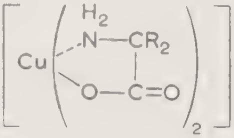
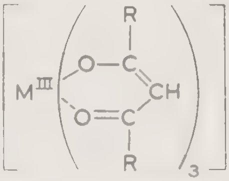
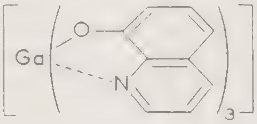
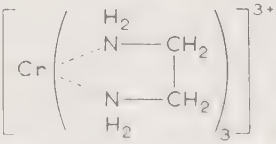
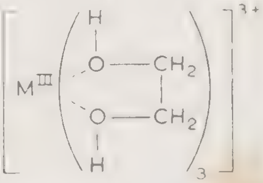
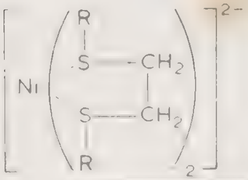
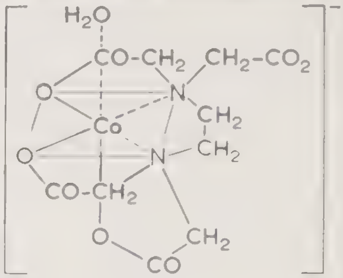
Chelating Group Classification	Chelating Group Examples	Typical Chelate Compounds Formed
1. Two acidic groups		
Dibasic inorganic acids	CO_3^{2-}, SO_3^{2-}	$[Co(NH_3)_4(CO_3)]^+$
Dicarboxylic organic acids	$C_2O_4^{2-}$, Phthalate	$[M^{III}(C_2O_4)_3]^{3-}$
α-Hydroxycarboxylic acids	Salicylate	
2. One acidic group, one coordinating group		
α-Aminocarboxylic acids	$R_2C(NH_2)CO_2^-$	 Cu H2 N—CR2 O—C=O 2
β-Hydroxy carbonyl compounds	1,3-Diketones, *o*-Hydroxyaldehydes	 M R O—C CH O=C R 3
8-Quinolinols	8-Quinolinol	
3. Two coordinating groups		
Diamines	Ethylenediamine	 Cr H2 N—CH2 N—CH2 H2 3 3+
Dihydroxy compounds	1,2-Glycols	 M H O—CH2 O—CH2 H 3 3+
Disulfides	Dithio ethers	 Ni R S—CH2 S—CH2 R 2 2−

TABLE I (continued)

Typical Chelating Groups

Chelating Group		Typical Chelate
Classification	Examples	Compounds Formed
4. Several acidic groups, two coordinating groups		
Diaminoacetic acids	ethylenediamine tetraacetate	

Diehl's (1937) distinction between anionic (*acidic groups*) and neutral (*coordinating groups*) donors is followed. Arrangements permitting the formation of five- or six-membered heterocyclic rings are most important.

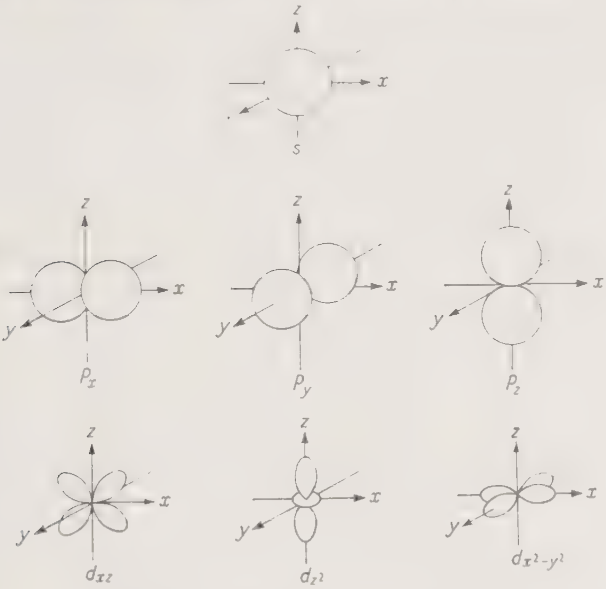
Acceptor cations are usually those of transition elements (e.g., V^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Co^{3+}, Ni^{2+}, Pd^{2+}, Pt^{4+}, Cu^{2+}, Ag^{+}, Zn^{2+}) or elements immediately following the transition elements in atomic number (e.g., Ga^{3+}, In^{3+}, Ge^{4+}). These ions all combine small size, large effective charge, and available *d* orbitals (p. 105) for bond formation. The lanthanide, actinide, and alkaline earth metal ions are poorer and less common acceptors. Alkali metal ions seldom form chelate rings.

B. Bonding

Species containing chelate rings vary in properties from relatively stable, highly covalent substances to very unstable, highly ionic substances. It is probable, therefore, that no single approach can explain adequately the bonding in all types of chelate compounds. For some, the covalent or electron pair bond gives the more logical explanation; for others, the ionic or electrostatic bond appears more reasonable. The general aspects of these two types of bonding are reviewed, respectively, by Keller and Parry (1956) and by Parry and Keller (1956). The entire subject of bonding is discussed critically by Coulson (1952).

1. *The Electron Pair Bond*

The original concept that the bond between a ligand and a cation involves donation of an electron pair from the former to the latter, although useful in elucidating the chemistry of these species, is untenable because of its implication of stationary electrons and negative charge accumulation on the cation. However, applications of the wave characteristics of the electron and of statistical probability in fixing its position have given quantum mechanical meaning to this concept (Coulson, 1952). An important conclusion involves the most probable spatial distribution of bonding s, p, and d orbitals as shown in Fig. 1. Both atomic and molecular orbital approximations have been employed to transfer this directional concept to chelate structures.



(Also d_{yz} and d_{xy} are similar to d_{xz}.)

FIG. 1. Directional characteristics of s, p, and d orbitals. Regions of most probable electron density with respect to x, y, and z axes.

In the atomic orbital approximation, it is assumed that an electron pair bond results when two atoms or ions, each containing a suitable unpaired electron, are brought together in such a way that appropriate unfilled atomic orbitals can interact by overlapping. The spatial distribution of atomic orbitals thus leads to directed bonds. Absence of sufficient orbitals in many cations requires that the normal arrangement be altered (excited) in favor of a new arrangement of sufficient equivalent bonding orbitals. The result is a type of *hybridization* and gives enhanced bond strength (Pauling, 1944). Hybrids invariably involve orbitals of nearly the same energy, sometimes s and p, sometimes s, p, and d. Thus, using small boxes to represent orbitals and arrows pointing down or up to represent electrons of different spin, one obtains for various cobalt species, as typical, the conditions shown in Fig. 2. Bonding in the $[Co(C_2O_4)_3]^{3-}$ ion is thus termed a d^2sp^3 hybrid. This hybridization is characteristic of all species with coordination number†

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† Coordination number, as applied to complex species, is the number of atoms bonded directly to a central metal atom.

6 and gives an octahedral spatial arrangement of donor groups about the central cation. Other common arrangements are sp^3 (tetrahedral) and dsp^2 (planar). Taube (1952) distinguishes between *inner orbital* complexes (principal quantum number of *d* electrons one less than that of *s* and *p* electrons) and *outer orbital* complexes (*d*, *s*, and *p* electrons all with same principal quantum number). The latter are more labile with respect to stability and exchange of ligands.

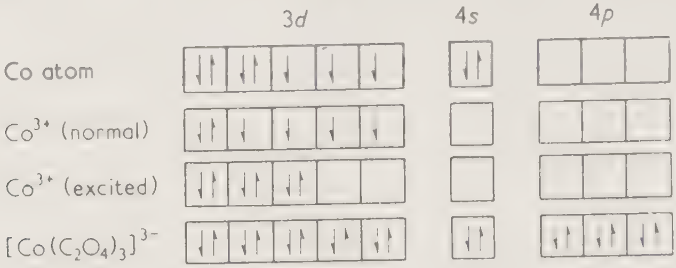


FIG. 2. Atomic orbital representations for cobalt species.

In the molecular orbital approximation, the bonding orbitals for the entire complex species are assumed to participate in the formation of each bond. All bonds in the species are thus interrelated, and any alteration in one bond will be communicated to all others. This is a definite advantage over the atomic orbital approach where each bond is considered as independent of all others. In addition, the molecular orbital approach offers a somewhat more realistic explanation of the multiple bonding found in some chelate species and permits the inclusion of species the properties of which are too ionic to allow their description in terms of atomic orbitals alone. A more exacting and extensive treatment is given by Coulson (1952).

2. *The Electrostatic Bond*

Early postulations that bonding in complex species results from electrostatic attraction between cations and either anions or dipolar molecules (e.g., water) were in keeping with qualitative observations that small, highly charged cations form complexes most readily, but they provided no quantitative correlations with the observed stabilities of the species and accounted for none of the many exceptions. Fajans (1928) first suggested ion deformation and polarization as necessary modifications to such a picture. The electron cloud of any ion or atom is deformed by the fields established by neighboring ions or dipolar molecules. The more loosely the electron cloud is bound to the nucleus, the more extensive the deformation. Such deformation promotes attraction between the two species and may be sufficiently large to impart to the complex so-called covalent characteristics. The broadened concept of electrostatic attraction thus embraces substances varying in properties from purely ionic to purely covalent. As such, it is attractive in describing chelate compounds. For transition metal complexes, these ideas have been expanded into the *crystal field* or *ligand bond theory* (Orgel, 1952, 1955, 1956; Nyholm, 1956; Moffitt and Ballhausen, 1956) which successfully employs the splitting of normally degenerate *d* levels as a result of electrostatic fields imposed by ligands to account for observed light absorption and magnetic effects. The spatial orientations of *d* orbitals as indicated in Fig. 1 indicate probable concentration of electron pairs along diagonals between the x, y, and z axes (as is found in octahedral complexes) as well as concentration along the three orthogonal axes (d_{z^2} and $d_{x^2-y^2}$). The crystal field approach is concerned with these spatial relationships and the probability that electrons will always give arrangements of minimum electrostatic potential energy.

C. Spatial Considerations

Synthesis, isolation of stereoisomers, resolution of optical isomers, and crystal structure determinations have all confirmed Werner's original postulations of octahedral distribution of ligands for coordination number 6 and of tetrahedral or planar distribution for coordination number 4. The ideas of hybridization agree with these results for covalent species. The crystal field theory suggests that the spatial arrangement in any specific substance is the one where repulsions among added groups are minimal. Again there is excellent agreement with observation.

III. Stabilities of Chelate Structures

A. Definition of Stability

For the equilibria

$$M + A \rightleftarrows MA$$

$$MA + A \rightleftarrows MA_2$$

$$MA_{n-1} + A \rightleftarrows MA_n$$

involving interaction of a central metal ion M with 1 to n donor molecules or anions A, the stability or formation constants of the resulting complex species are defined by the equations

$$K_1 = \frac{(MA)}{(M)(A)} \tag{1}$$

$$K_2 = \frac{(MA_2)}{(MA)(A)} \tag{2}$$

$$K_3 = \frac{(MA_n)}{(MA_{n-1})(A)} \tag{3}$$

where the parentheses represent the activity of the species shown. Since activity is given by the product of the molar concentration and an activity coefficient f, Eq. (3) may be expressed

$$K_n = \frac{[MA_n]}{[MA_{n-1}][A]} \times \frac{f_{MA_n}}{f_{MA_{n-1}} f_A} \tag{4}$$

where the brackets represent the concentration of the individual species. The "concentration stability constant" K_{cn} is defined as

$$K_{cn} = \frac{[MA_n]}{[MA_{n-1}][A]} \tag{5}$$

Hence, Eq. (4) may be expressed as

$$K_n = K_{cn} \frac{f_{MA_n}}{f_{MA_{n-1}} f_A} \tag{6}$$

Most of the stability constants currently listed for chelate compounds have been measured in a controlled ionic medium (usually at ionic strength 0.10) and are thus concentration stability constants.

B. Factors Affecting the Stabilities of Chelate Structures

Such factors are related either to the effect of the structure of the chelating agent or to the effect of the metal ion.

1. *Structure of the Chelating Agent*

The effect of structure of the chelating agent is determined by ring size, polydentate character, ligand basicity, nature of the donor atom, resonance effect, and steric effects. These are discussed comprehensively by Martell and Calvin (1952, see pp. 134–180) and Parry (1956).

a. Ring Size. As suggested previously, the most stable chelate rings have five or six members. No well-authenticated example of a three-membered ring is known. Four-membered rings are uncommon but are known in carbonato complexes (e.g., $[Co(en)_2CO_3]^+$), in the chloro bridges in the compound Al_2Cl_6, and in ethyldithiocarbamate complexes of nickel(II). Very few compounds with seven-, eight-, or ninemembered rings have been described. These are invariably prepared in nonaqueous media because the tendency toward chelation is so slight that in aqueous solution competition between water and the ligand for the metal ion favors the aquo (water) complex.

When a saturated ligand forms a five-membered ring with a given cation, the resultant chelate is more stable than when a similar saturated ligand forms a six-membered ring. Thus, glycine forms more stable chelate compounds than β-alanine. However, if the ligand contains two double bonds, the six-membered ring is usually the more stable. Thus, chelate compounds derived from 1,3-diketones and salicylaldehyde are very stable.

Schubert and Lindenbaum (1952) have compared the formation constants of the calcium and strontium complexes of twenty-five mono-, di-, and tricarboxylic acids. Certain of these acids form four-membered or seven-membered rings. However, when the structure of the polycarboxylic acid is such that the formation of a five- or six-membered ring is possible, the stability of the complex is increased, e.g., the calcium-citric acid chelate. However, direct correlation between ring size and formation constant is possible only when the basicity of the ligand, the nature of the donor atom, polydentate character, and resonance effects are all held constant.

b. Polydentate Character. Martell and Calvin (1952, see pp. 143–147) have presented quantitative data which prove conclusively that an increase in the number of rings in a particular structure is paralleled by an increase in stability of the species. Examples of this effect and an explanation are given in the section on thermodynamic considerations.

c. Ligand Basicity. Factors governing binding of an acidic group to a metal ion are similar to those governing binding of such a group to a proton. Thus, the greater the basicity of the donor group, the greater the stability of the chelate compound. Direct correlation between basicity of the ligand and stability constant results, however, only when ring size, polydentate character, resonance effect, and steric effects are held constant.

d. Nature of the Donor Atom. This effect can be discussed only qualitatively, for change in the donor atom is invariably accompanied by change in base strength, steric factors, ring size, etc. Only nitrogen, oxygen, and sulfur donors need be discussed. With both nitrogen and oxygen, the tendency toward coordination decreases with increasing substitution; whereas with sulfur, the reverse is true:

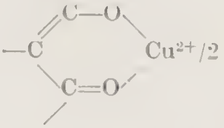
$$H_2O > ROH > R_2O$$

$$H_3N > RNH_2 > R_2NH > R_3N$$

$$R_2S > RSH > H_2S$$

Metal ions differ in their preference for coordination to nitrogen or oxygen or to oxygen or sulfur. Ions preferring nitrogen to oxygen are Cu^+, Ag^+, Au^+, Cu^{2+}, Cd^{2+}, Hg^{2+}, V^{3+}, Co^{3+}, and Ni^{2+}. Those showing little preference are Be^{2+}, Cr^{3+}, Fe^{2+}, and platinum metal species. Other cations prefer oxygen to nitrogen. Ions such as Cu^+, Ag^+, Au^+, and $Hg_2{}^{2+}$ prefer sulfur to oxygen, whereas the ions Be^{2+}, Cu^{2+}, Au^{3+}, and most others prefer oxygen to sulfur. There is at present no logical explanation for these observations.

e. Resonance Effect. This may either decrease or increase the stability of the chelate species depending upon whether resonance within the chelate ring is interfered with or enhanced by the over-all structure of the species. The extraordinary stability of metal derivatives of porphyrin and phthalocyanin may be explained if one assumes that the metal-nitrogen bond has some of the characteristics of a double bond. The resulting chelate then has a completely conjugated system of double bonds. On the other hand, participation of the chelate ring in resonance of the attached groups reduces stability. Thus, the stability of the copper(II)-enolate ring



decreases on substitution of salicylaldehyde for acetylacetone because resonance in this ring is disturbed by participation in resonance of the attached benzene ring.

f. Steric Effects. An attached group on the ligand may interfere sterically with chelation and thus reduce stability. Thus, the stability of the *C*-methylbenzoylacetone chelate of copper(II) is much less than one would expect on the basis of basicity and resonance effects. It appears that the chelate ring is distorted from the planar arrangement essential for complete resonance by steric hindrance between the methyl group and the ortho hydrogen atoms of the benzene ring. Other examples of steric effects are the following: 6,6′-dimethyl-2,2′-dipyridyl does not give chelate rings with iron(II) ion, whereas 2,2′-dipyridyl does; 2-substituted 8-quinolinols do not give chelate rings with aluminum ion, but 8-quinolinol does.

2. *The Effect of the Metal Ion (Empirical Correlations)*

For chelate compounds that are primarily ionic, such as those of the lanthanide and alkaline earth metal ions, and for a given chelating agent, stability may be expected to increase with increasing ionic charge and decreasing cation radius. However, the energy of hydration of a spherical gaseous ion is proportional to the charge squared divided by the radius (i.e., to Z^2/r). Since in chelate ring formation the hydration sphere of the cation is partially or completely displaced, stability should be more closely related to the quantity Z^2/r than to the simple charge to size ratio. Martell and Calvin (1952, see pp. 181–206) have shown that for the alkaline earth metal ions, log K_{cn} and Z^2/r are related linearly for a variety of chelating agents, except for frequent maxima in log K_{cn} at calcium. A similar linear relationship is found for ethylenediaminetetraacetate and 1,2-diaminocyclohexanetetraacetate chelates of the lanthanide ions, but with the similar *N*-hydroxyethylethylenediaminetriacetate species, the relationship fails in the middle of the lanthanide series. Although cation size and charge influence stability among these ionic complexes, they are apparently not the only factors involved.

Martell (1955a), in an attempt to correlate the tendencies of cations to form chelate rings with a single linear function, has shown that the stability constants of dibenzoylmethane compounds with metal ions of the same charge vary linearly with the electronegativity of the ion and that the slopes of the lines in such a plot increase with increasing cation charge. On the assumptions that univalent cations bond through *s* orbitals, bivalent (except Pd^{2+} which bonds through dsp^2) through sp^3, and trivalent through d^2sp^3 and that the relative bond strengths are 1.0 (*s*), 2.0 (sp^3), 2.7 (dsp^2), and 3.0 (d^2sp^3), he has found further that plots of log K_{cn} vs. the product of electronegativity and bond strength are approximately linear. Calvin (1954) has shown that ionization potential and electronegativity bond strength product are equivalent and that plots of log K_{cn} vs. ionization potential are approximately linear for a variety of cations. The theoretical significance of such empirical correlations is not immediately apparent.

Data on stability constants indicate the following orders of decreasing stability with a given chelating agent:

$Pd^{2+} > Cu^{2+} > Ni^{2+} > Pb^{2+} > Co^{2+} > Zn^{2+} > Cd^{2+} > Mg^{2+}$

$Cu^{2+} > Ni^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+}$

$Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+} > Ra^{2+}$ (Ca^{2+} and Mg^{2+} are often inverted)

$Tl^{3+} > Fe^{3+} \geqq Ga^{3+} > In^{3+} \geqq Al^{3+} > Cr^{3+} > Sc^{3+} > Ln^{3}$

$Lu^{3+} > Tm^{3+} > Y^{3+} > Gd^{3+} > Sm^{3+} > Nd^{3+} > La^{3+}$ (Y^{3+} sometimes displaced to Nd^{3+} position)

Such arrangements are only average, and a given cation may change its position as a result of changes in steric requirements, nature of the donor atom, etc.

C. Thermodynamic Considerations

Evaluation of changes in standard free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) for a number of reactions yielding chelate species has aided in understanding these substances. Such thermodynamic constants are most commonly calculated from thermodynamic stability constants measured at several temperatures by means of the equations

$$\Delta F^\circ = -RT \ln K \tag{7}$$

$$\frac{d \ln K}{dT} = \frac{\Delta H^\circ}{RT^2} \tag{8}$$

$$\Delta F^\circ = \Delta H^\circ - T\Delta S^\circ \tag{9}$$

Occasionally, ΔH° can be measured by a direct calorimetric method (cf. Vol. I, Chapter 2 for a more detailed discussion of these relationships).

The stability of a complex species is related to the difference between its free energy content and the free energies of its dissociation products. Both the metal-donor bond energy and the entropy change contribute to the decrease in free energy during formation of the species. The relative contributions of the two depend on the metal ion and the complexing agent involved.

Schwarzenbach (1954) has ascribed the greater stabilities of chelate compounds over those formed by monodentate ligands to entropy changes (i.e., to increases in the disorder of the system). Complex species are formed from hydrated cations in aqueous systems by displacement of water molecules. If the ligand is monodentate, the number of water molecules displaced is the same as the number of ligands bonding to the cation, and there is no change in the entropy of the system. This is shown in the equation

$$Zn(H_2O)_4{}^{2+} + 4NH_3 \rightleftarrows Zn(NH_3)_4{}^{2+} + 4H_2O$$

If the ligand is bidentate, however, two water molecules are replaced by each molecule, and the total number of free particles increases. This is shown in the equation (en = ethylenediamine)

$$Zn(H_2O)_4{}^{2+} + 2en \rightleftarrows Zn(en)_2{}^{2+} + 4H_2O$$

In these two reactions, the zinc-nitrogen bond energies are nearly the same, and the changes in enthalpy are almost identical. Thus, the greater stability of the chelate species, as reflected in a larger stability constant, is due almost entirely to an increase in entropy. This is made more quantitative by applying Eqs. (7), (8), and (9) to these reactions as

$$\Delta F_1^\circ = \Delta H_1^\circ - T\Delta S_1^\circ = -RT \ln K_1$$

$$\Delta F_2^\circ = \Delta H_2^\circ - T\Delta S_2^\circ = -RT \ln K_2$$

or since $\Delta H_1^\circ = \Delta H_2^\circ$

$$\Delta S_2^\circ - \Delta S_1^\circ = R(\ln K_2/K_1) \tag{10}$$

where subscript 1 refers to the ammonia system and subscript 2 to the ethylenediamine. The larger the number of rings formed, the greater the increase in number of particles and in resultant entropy change.

Parry (1956, see p. 251) has tabulated thermodynamic quantities for equilibria of the type

$$MA_2{}^{2+} + \text{en} \rightleftarrows M(\text{en})^{2+} + 2A$$

where A represents ammonia or methylamine and M a divalent cation. For the cadmium-methylamine reaction and the cadmium- and zinc-ammonia reactions, the enthalpy changes are very small and the increase in stability is due primarily to increase in entropy.

Occasionally, however, as in reactions involving $Cu(NH_3)_2{}^{2+}$ ion and ethylenediamine (en), changes in enthalpy ($\Delta H = -2.6$ kcal) become significant in contributing to increased stability ($\Delta F = -4.3$ kcal). This appears to be caused by a reduction in repulsive forces brought about by the replacement of two monodentate groups by a bidentate group (Williams, 1954).

Carini and Martell (1954) have evaluated these thermodynamic constants for the formation of alkaline-earth metal-ethylenediaminetetraacetate complexes. Changes in standard enthalpy are small and negative and are of nearly the same magnitude regardless of cation. Numerically the values are small compared to heats of hydration of the gaseous ions and approximate the magnitudes expected for ion-dipole bonds involving hydrated ions. The relatively large stabilities of the complexes are due, therefore, to large entropy increases during their formation. For example, with the calcium complex, the entropy change is about four-fifths the free energy change. Changes in entropy are due to decrease in ionic charge, increase in size of ionic species, and increase in the number of particles. The last effect is believed to be the most important.

Available thermodynamic quantities are listed by Martell (1955b) and by Williams (1954).

D. Determination of Formation Constants

Because of space limitations, this topic is best covered by references. Martell and Calvin (1952, see pp. 76–133) discuss comprehensively most of the available methods. Schwarzenbach *et al.* (1954) and Schmid and Reilley (1956) describe methods applicable to very stable species. Schubert (1954a) gives a new biological method.

An up-to-date and complete tabulation of constants has been compiled by Bjerrum *et al.* (1957, 1958).

E. Points to Consider When Applying Data on Stability Constants

The following considerations may be helpful when it becomes necessary to extend observations to conditions which differ from those under which the original measurements were made :

1. *Dielectric Constant of the Solvent System.*

Presently available information is insufficient to permit evaluation of the effect of dielectric constant on the stability of a chelate. One should not try to apply data obtained for one solvent to another solvent.

2. *Ionic Strength*

Stability constants may vary in magnitude by a factor of ten with changes in ionic strength, even though concentration remains constant. Conversion of a value from one ionic strength to another requires evaluation of activity coefficients. This can seldom be done with complete accuracy for the chelate species involved. It is possible to predict variations with some accuracy, however, by calculating the activity coefficients of the ions involved by means of the Debye-Hückel equation (cf. Izatt, *et al.*, 1954).

3. *Competing Ions*

a. Hydrogen and Hydroxyl Ions. At low pH, hydrogen ions compete with metal ions for donor groups, whereas at high pH, hydroxyl ions compete with donor groups for metal ions. These effects are of particular importance when one is concerned with the quantities of uncomplexed metal ion present at various pH values. The stability constant is not necessarily an index of the ability of a chelating agent to tie up metal ions. This can be shown qualitatively by considering the reactions of two different chelating agents, A^- and B^-, with the ion M^{2+}, as

$$M^{2+} + A^- \rightleftarrows MA^+$$

$$M^{2+} + B^- \rightleftarrows MB^+$$

The expressions for the stability constants, assuming equality between concentration and activity for simplicity, are then

$$K_A = \frac{[MA^+]}{[M^{2+}][A^-]} \quad \text{and} \quad K_B = \frac{[MB^+]}{[M^{2+}][B^-]} \tag{11}$$

If one assumes that $K_B > K_A$ and that the chelating agent B^- is much more basic than A^-, it is apparent that at low pH, A^- binds a greater percentage of M^{2+}. The amount depends upon the exact values of K_B and K_A, for hydrogen ions decrease the concentration of B^- more than that of A^-. For example, Schubert (1954b) has shown that K_1 for the manganese-proline chelate is three times as great as K_1 for the manganese-glycine chelate. However, at pH 8.0, glycine complexes nearly three times as much Mn^{2+} because of its greater acidity and the thus higher concentration of chelating anion present. Therefore, for comparisons among chelating agents it is necessary to calculate the concentration of the particular chelating species to be used in the stability constant expression at the specific pH employed.

At high pH, one must take into account precipitation of hydroxides. Consideration of the concentration of hydroxyl ion, the solubility product constant of the metal hydroxide, and the concentration of uncomplexed metal ion indicates whether the complex is sufficiently stable to prevent precipitation of the hydroxide. A metal may be inert physiologically, even though it forms very stable chelates, if it also forms a very slightly soluble hydroxide. Zirconium, for example, is practically inert physiologically because of the extremely small solubility of zirconium hydroxide at pH 7.

b. Anions. Certain anions will precipitate insoluble metal salts if the chelated species is not sufficiently stable. Evaluation of the concentration of uncomplexed metal ion from the stability constant and the pH indicates whether the solubility product constant of that salt is exceeded. For example, the calcium-ethylenediaminetetraacetate chelate is sufficiently stable ($\log K = 10.7$) to prevent precipitation of calcium carbonate ($\log K_{sp} = -8$) at pH 7. Thus, ethylenediaminetetraacetate salts are used to dissolve calcium deposits in the body.

c. Cations. A second metal ion that can form a chelate with the reagent in question will cause competition for the chelating agent. The extent of the displacement or competition reaction depends upon the relative stabilities of the two chelates. Thus, for a displacement equilibrium

$$M_1 + M_2A \rightleftarrows M_2 + M_1A$$

where M_1 and M_2 are two different metal ions and A is a chelating agent, the equilibrium constant K is

$$K = \frac{[M_2][M_1A]}{[M_1][M_2A]} = \frac{K_{M_1A}}{K_{M_2A}} \tag{12}$$

where K_{M_1A} and K_{M_2A} are the respective stability constants. This type of displacement reaction occurs frequently in biological systems. For example, Schubert (1954c) explains the inability of calcium ions to reverse beryllium inhibition of alkaline phosphatase in terms of the small value of the ratio of the stability constant of the calcium complex with the phosphatase to the stability constant of the corresponding beryllium complex. If high (unphysiological) concentrations of calcium ions were employed, then reversal could be effected. On the other hand, divalent cobalt and nickel, which form strong complexes with the same groupings as beryllium, have the power to reverse beryllium inhibition of alkaline phosphatase at reasonable concentration levels.

d. Chelating Anions. A second chelating agent will compete for the metal ion. Thus, for the equilibrium

$$MA + B \rightleftarrows MB + A$$

where A and B are two different chelating agents and M is a metal ion, the equilibrium constant K is

$$K = \frac{[MB][A]}{[MA][B]} = \frac{K_{MB}}{K_{MA}} \tag{13}$$

where K_{MB} and K_{MA} are the respective stability constants. An example of this type of displacement reaction is the reaction of the salicylate ion with complexed beryllium. The salicylate ion has the ability to reverse beryllium inhibition of alkaline phosphatase (Schubert, 1954c), forming a very stable chelate with beryllium at physiological pH and, when present in sufficient concentration, removing the beryllium from the alkaline phosphatase chelate.

e. Temperature. If the enthalpy change (ΔH°) is known, change in stability constant with temperature can be calculated from Eq. (8).

IV. Conclusion

In this chapter, emphasis has been placed upon the properties of chelate structures and upon the various factors influencing the chelation process. Although specific and detailed information about the biological implications of the process has not been given, it is probable that the same principles and characteristics apply to these systems and that chelation as it relates to the behavior of inorganic ions is no different with biological materials from what it is with nonbiological materials.

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CHAPTER 5

Compartmental Methods of Kinetic Analysis†

A. K. SOLOMON

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I. Introduction

A. Scope and Value of Compartmental Analysis

Compartmental analysis of biological systems is concerned primarily with the development of abstract models which reproduce the kinetics of tracer distribution in biological systems. The strength of the method lies in the power of abstraction, and its weakness comes from the fact that these models cannot yet be complete representations of entire biological systems. When a radioactive or stable isotopic tracer is injected into a biological system, it produces a perturbation in the isotopic constitution of one chemical species. The effect of this perturbation is measured by observing the subsequent fate and equilibrium distribution of the isotope. Compartmental analysis is then used to synthesize the simplest model which can simulate the observed kinetic behavior of the tracer. This initial step, the exact description of a gross process, is the first stage of classification. The simple model may then be subject to further experimental test to determine how well it succeeds in providing an accurate representation of the biological system. The final resolution of the problem requires an understanding of each reaction or process involved in the distribution of the tracer.

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The term *compartment* is used in a double sense. On the one hand it refers to a physiologically realizable region of space bounded by membranes or other barriers which limit the region. Examples of such physiological compartments are the plasma, the red blood cell, a single muscle cell or a group of muscle cells, a single neuron or the entire brain. The physiological compartment is loosely analogous to a phase. In thermodynamic terms, a phase is usually homogeneous in composition in the sense that a small element of volume taken from part of the phase will be indistinguishable from a small element taken from another part of the phase. However, on the molecular level there are sharp discontinuities between one molecule of solute and a directly adjacent molecule of solvent. It is frequently convenient to neglect these differences on the molecular level and describe the behavior of the phase in terms of a uniform assembly. It is understood that the behavior of the phase is governed by the behavior of all of the individual molecules and ions that make up the phase. Similarly, when a whole cell is considered to be a *compartment*, it is recognized that a number of subcellular entities are present in the compartment. It is frequently convenient to neglect these entities and to treat the cell or the assembly of cells as a single homogeneous unit. In this case, too, the behavior of the compartment is governed by the behavior of the individual molecules and ions that make up the cells. The difference lies in the level of approximation, which is much grosser for a physiological compartment than for a thermodynamic phase. It is a reflection of the complexity inherent in living matter.

The second sense in which compartment is used is the chemical one. When a compound is synthesized in a biological system, the compound itself may be considered as a compartment, and the same treatment can be applied to chemical as to physiological compartments. Examples of chemical processes which will be discussed in detail are the synthesis of cholesterol in man from the hydrogen in body water and the synthesis of phospholipid in the liver from circulating phosphate. The pool of exchangeable cholesterol in the entire body is considered to constitute a compartment, as is the total liver phospholipid. In the case of a chemical compartment, the homogeneity is purely that of a molecular species, and there is no identification with a region of space.

It is convenient at this point to introduce two definitions of terms commonly used to describe the rate of a process. The *flux* of a substance from one compartment to another is defined as the amount of substance which crosses the boundary in unit time. In physiological compartments, it is also necessary to specify the area across which the flux takes place, so that units of amount $\times$ time^{-1} $\times$ area^{-1} are used. In chemical compartments, flux is expressed in units of amount $\times$ time^{-1}. An alternate method of description of the rate of the process is by means of the *rate constant*, which is the flux per unit amount of substance in the compartment *from* which the substance flows. Thus the rate constant has units of (time $\times$ area)$^{-1}$ or (time)$^{-1}$.

Even in the apparently simple situation in which a molecule is transported from one side of a cellular membrane to another, a host of complex problems must be considered. The over-all rates of entrance of many test substances into human red cells have been measured and characterized experimentally by assigning a definite value to the flux of test substance into the cell. On a molecular basis, however, this journey from the outside of the membrane is complicated by the solubility of the test substance in the various components that make up the membrane, by the geometry of the membrane, by the quantity of water in the membrane; in sum, by all of the interactions of the entering molecules with the lipids, proteins, and water that make up the membrane. In this case, flux is the cover word used to describe the abstraction involved in denoting the process by a single rate constant. When a membrane is replaced by a cell, the problem becomes more complex since the probing molecule must traverse two membranes and a cell interior. As the cell grows into a more complex structure, as for example the intestinal mucosa, the model becomes even more abstract. Nonetheless, the concept of the model is of great importance since it provides a method of grasping the essentials of the problem. Furthermore, it circumscribes the ultimate solution since, if the abstraction is correct, the final response of the system when all the molecular interactions have been determined will be equivalent to the response of the model system.

Many examples can be found in the literature of applications of similar forms of analysis to biological problems as, for example, in pharmacology and in studies of the kinetics of chains of biochemical reactions (Teorell, 1937*a*, *b*; Hearon, 1949*a*, *b*). The present chapter will be concerned, however, with a more limited problem, namely, the use of compartmental analysis in interpreting the results of experiments on biological systems with radioactive and stable tracers. It will be illustrated with numerous examples, many taken from the author's laboratory since these are the most familiar to him. Many general treatments of compartmental analysis of tracer systems are available in the literature as, for example, those of Sheppard (1948), Solomon (1949, 1953), Sheppard and Householder (1951), and Reiner (1953). These have recently been reviewed by Robertson (1957).

B. Validity of Assumptions Usually Made

The following assumptions are usually inherent in the use of compartmental analysis in the treatment of tracer kinetics.

1. The composition of each compartment is uniform, and the resistance to flow between compartments is localized in a single sharp step at the interface between compartments.
2. The injection of the tracer does not affect the behavior of the system.
3. The behavior of the labeled molecule is exactly like that of the unlabeled molecule, and the system cannot discriminate between them.
4. Movement into or out of a compartment results from a random process.

The single most important assumption is the localization of the resistance to tracer flow to the interface between compartments. This means that all other reactions in the system are so fast that they may be neglected in comparison with the slow step involved in crossing the compartmental boundary. When one step in a sequence of reactions is much slower than any of the other steps, its rate determines the forward velocity of the whole sequence, and it is called the *rate-limiting* reaction. Consequently, the requirement that the rate-limiting step be at the interface places an effective limit on the inhomogeneity that may be tolerated in a compartment. Mixing within a compartment, following the introduction of tracer, must be fast enough to ensure uniform distribution throughout the compartment before any appreciable amount of tracer leaves. If the rate-limiting step is slow, mixing may be sluggish ; if it is fast, mixing must be even faster. Once this requirement is satisfied, the driving force within the compartment may be assumed to be uniform, as is required by the ordinary equations of compartmental analysis.

When whole cells represent a single compartment, it is necessary to be sure that the viscosity of the cellular contents and the presence of intracellular barriers do not make the assumption of a uniform compartment untenable. Similarly when material is brought to the rate-limiting membrane by the blood supply, it is necessary to ensure that the process is limited by movement across the membrane, and not by the rate of supply of material to the membrane. In this connection, Pappenheimer and associates (1951) have pointed out that there is an important concentration gradient for diffusible substances along the length of the capillary and that the assumption that the concentration of a substance is constant in a capillary bed is not generally acceptable. The kinetics of diffusion in biological systems has been thoroughly treated by Jacobs (1935) in his great monograph, and problems in which both diffusion and transport across membranes affect the over-all rate have been solved by Hearon (1950). The present chapter will be limited to a discussion of situations in which diffusion does not appear to affect the rate-limiting step.

The localization of the resistance in a single barrier raises several interesting problems. As a molecule moves from the center of a well-mixed bulk solution to the center of the membrane, it encounters regions in which the membrane encroaches into the domain of the solution, and then enters other regions in which the solution appears to encroach into the domain of the membrane. Compartmental analysis cannot yet resolve membrane structure in these terms, since so little is known of the fine structure of membranes. However, the membrane phase may in some cases constitute a compartment, provided the amount of tracer contained in the membrane is appreciable and the rate of movement into or out of the membrane compartment is slow compared to the rate of flow into or out of the bulk solution.

In order for tracers to be used satisfactorily in compartmental analysis, it is necessary that the injection of the tracer leave the system undisturbed. In particular, the tracer can only be accompanied by a limited amount of unlabeled isotope, which usually requires high specific activity.† If it is essential that the addition of the tracer change the concentration of the marked element, it is necessary to make sure that the initial disturbance has subsided before making measurements that are interpreted as characteristic of the steady state. The amount of radiation delivered to the system must be controlled to avoid direct radiation effects.

The basis for the separation of isotopes by chemical processes is the difference in reaction rates between molecules of different isotopic composition. These differences may be quite appreciable. Bigeleisen (1949) has calculated that the reaction rates of C^{12}-containing radicals may be 1.25 times greater than the reaction rates for the same radical labeled with C^{14}. As the percentage difference in atomic weight decreases, the importance of the isotope effect diminishes ; the factor is calculated to be 1.02 when P^{31} is compared with P^{32}. A similar isotope effect has been observed in diffusion measurements. Thus, the diffusion of water measured using THO is 14% slower than it is when measured using H_2O^{18} (Wang *et al.*, 1953). Since the percentage change in the weight of the marked atom is much less when O^{18} is used as a tracer than when T is used, the measurements with H_2O^{18} are considered to be more representative of the behavior of unlabeled water.

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† Specific activity is defined as the relative number of tracer molecules (or atoms) per unit amount of chemical species.

It is generally assumed that the rate of formation and destruction of a labeled molecule is a random process, independent of the age of the molecule or the age of the cell in which the molecule is contained. However, red cell hemoglobin formation and destruction is not consistent with this assumption and may not be treated by the ordinary equations of compartmental analysis, since Shemin and Rittenberg (1946) have shown that hemoglobin, once formed, remains intact within the red cell during the 127-day life span of these cells. These authors have developed a special and elegant mathematical description of tracer behavior under such conditions.

C. The Steady State and the Driving Forces

Ideally, a system studied with the aid of tracers should be in the steady state. The steady state is characterized by compartments which are constant with respect to composition and volume, though there may be a flow of material across the compartment. Fresh reactants are supplied to the system at a constant rate and reaction products leave the system at a constant rate. The amount of substance in a compartment in the steady state remains constant, though the identity of the individual molecules, and hence the tracer concentration, is constantly changing. However, the steady state need not represent a condition of thermodynamic equilibrium,† since energy is usually dissipated to support it.

There are important instances in which the steady state may not be maintained. Thus, isolation of the system may cause it to leave the steady state, owing to injury, the loss of substrate, or the accumulation of product. Such systems may also be studied with tracers and described analytically, provided the changes in composition can be described mathematically with soluble equations. It is always easier to study unsteady-state conditions when the changes in composition are slow compared with the flux of tracer, but this requirement is not essential.

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† In a closed system, chemical equilibrium is also a steady state and hence should rigorously be included in the definition of the steady state. In this case no energy is dissipated to support the equilibrium. Conventionally the term " steady state " is reserved for nonequilibrium conditions.

The driving force for net transport from one physiological compartment to another is usually the electrochemical potential gradient under conditions in which volume flow and magnetic and gravitational forces may be neglected. Similarly, in chemical reactions the forward driving force is usually the chemical activity of the reactants. In the steady-state condition these forces remain constant. When a tracer is added to a system in the steady state, the driving force for tracer movement becomes the specific activity of the tracer, that is, the relative number of tracer molecules per unit amount of chemical species (as, for example, counts per minute per millimole). In a closed system at infinite time, tracer distribution leads to equal specific activity in each compartment. Similarly, when a chemical process has come to equilibrium, the chemical activity of each component is the same in each phase.

As Robertson (1957) has pointed out, the equations governing tracer movement in compartmental analysis follow first-order reaction kinetics in the steady state. This is a consequence of the fact that the driving force for tracer distribution is the specific activity of the tracer and not the chemical activity of the reactants. As a result, in the steady state, tracer movement is independent of the actual order of the reaction being studied. In order to determine the reaction order, it is necessary to measure the tracer flux at a number of different reactant concentrations. It is not possible to establish the order of a chemical reaction without allowing the chemical concentration of the reactants to change.

One important point must be emphasized in treating heterogeneous systems. The net transfer from one compartment to another represents the movement of an *amount* of substance across a barrier. The forward driving force, however, is the specific activity within the compartment from which the flux originates. To convert an amount into a specific activity one must know the chemical concentration of the marked species (labeled plus unlabeled) and the volume of the compartment, that is, the content of marked species in the compartment. Consequently, the content of the compartment must be introduced explicitly into the calculations of transfer coefficients. This is an important difference between the usual chemical first-order reactions which take place in homogeneous systems, and compartmental analysis. In general, the simple analogies between conventional kinetic analytical methods and compartmental analysis are misleading, largely because of this difference. Conventional systems are usually homogeneous and occasionally, as in the case of radioactive decay, are concerned with irreversible reactions only. Intuition in heterogeneous sytems is a fallible guide ; sure conclusions follow only from the orderly development of the necessary descriptive equations.

D. Choice of Model System

The most satisfactory model is the one which requires the least number of compartments ; the guiding principle is economy. How is it possible to decide whether the minimum number of compartments is compatible with the system's behavior? It is certainly not sufficient merely to investigate the one-way flow of tracer into a compartment, for three-compartment systems may well masquerade as two-compartment ones if no more stringent test is applied. The assumption of two-compartment kinetics receives considerable support if tracer is added to the second compartment so that the flow of tracer in the reverse direction may also be studied. The flux measured in both directions must be the same, and it must be time-invariant. Alternately, if both compartments are accessible to experimental observation, it is possible to make sure that the sum of the amounts of tracer in both compartments is always equal to the initial amount of tracer added (after correction for radioactive decay). An independent check of the validity of the model is always an essential component of satisfactory compartmental analysis.

Compartments that do not affect a measurable rate-determining step are invisible and may often be neglected in the mathematical treatment. Thus an inaccessible fast compartment wholly contained within a slower compartment will escape detection. Fast compartments can hide behind slow compartments, and very slow compartments (an order of magnitude or two slower than the other compartments) usually will not affect the rate-determining step if they provide a parallel path. If such a slow parallel branch is present, the specific activities throughout the system will not all be equal when the tracer has apparently reached its final distribution. At true tracer equilibrium, which is not necessarily chemical equilibrium, the specific activity must be constant everywhere throughout the system. Consequently, measurement of tracer equilibrium specific activities provides an important test for the validity of the model. Although a slow parallel compartment will not distort the rate of the limiting step, its presence and size must be determined in order to express the driving force for the rate-limiting step correctly.

The biological identification of compartments also offers a considerable problem. Compartmental analysis only reveals the presence of barriers to tracer movement in the system ; it does not delimit the physical size of the compartments or specify the exact position of the barriers. This information, which comprises the physiological localization of the compartment, requires additional data in the form of samples taken from each compartment during the experiment. Since this procedure often introduces practical difficulties, identification remains one of the most difficult problems encountered in the course of compartmental analysis.

II. One- and Two-Compartment Systems

A. Symbols

Interest is usually centered on the determination of the flux, the amount of material that crosses a unit area of compartmental boundary in unit time, or alternately on the rate constant, which is the flux per unit amount of substance in the compartment *from* which the flux originates. The dependent variable in the equations developed may have units of amount, concentration, or specific activity. The choice between these is usually a matter of convenience, since the fluxes determined by the equations must be independent of the choice of variable. The following symbols will be used :

P_i = amount of tracer in Compartment i (counts per minute)
v_i = volume of Compartment i
$p_i = P_i/v_i$ = concentration of tracer in Compartment i (counts per minute per unit volume)
S_i = amount of substance (labeled plus unlabeled) in Compartment i
Φ_{12} = flux of substance from Compartment 1 to Compartment 2 (amount per unit time [and per unit area])
$k_{12} = \Phi_{12}/S_1$ = rate constant, fraction of S_1 transferred to Compartment 2 per unit time [and per unit area]
$p_i^* = P_i/S_i$ = specific activity of substance in Compartment i (counts per minute per millimole).

The subscripts 0 and ∞ are used to denote zero time and infinite time, respectively.

Important distinctions are to be made between steady and unsteady states, between open and closed systems, and between reversible and irreversible reactions. Open systems are those connected to either a source of tracer or a sink or both. In closed systems, the amount of tracer is conserved. Systems in which there is an irreversible step with respect to tracer movement behave as though they were open systems, and have been described by Robertson (1957) as systems without recycling of tracer. Considerable simplifications in mathematical treatment are possible in such cases. Thus, the equations developed by Bateman (1910) governing the synthesis and decay of radioactive elements in natural radioactive chains may be applied directly. Half-life has a clear and explicit meaning. However, the conclusions drawn from systems with an irreversible step may not be applied to reversible systems. This is a common error of intuition that arises because the kinetics of radioactive decay are so familiar.

B. One-Compartment System—Steady State

One-compartment systems are necessarily open systems and may be described by the kinetics of simple radioactive decay. Consider a one-compartment system in the steady state, containing tracer initially.

$$dP/dt = -\Phi p^* \tag{1}$$

Since

$$P = p^* S \tag{2}$$

and S remains constant in the steady state, the solution is

$$k = \Phi/S = -(\ln p^*/p_0^*)/t \tag{3}$$

Since $p^*/p_0^* = p/p_0 = P/P_0$ when S and v remain constant, as they do in the steady state, it does not matter which variable is chosen for measurement. In practice, if k alone is desired and the system has been shown to be in the steady state, it is usually most convenient to measure p, since this obviates the requirement for chemical assay for S, or any measure of v. For example, when D_2O is injected intravenously in man, its disappearance from the serum may be expressed in terms of a single exponential with a half-life of 11 days (Schloerb *et al.*, 1950) as shown in Fig. 1. In this case, mixing is probably completed in one hour, by

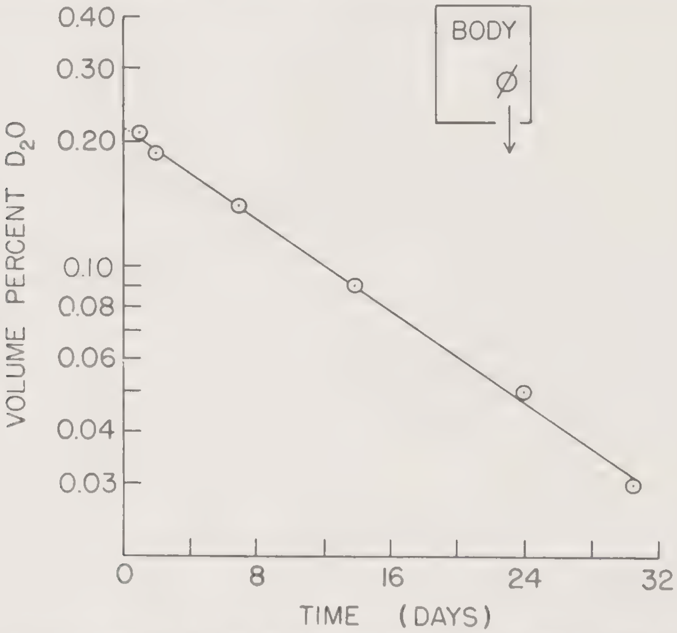

FIG. 1. Disappearance of D_2O from the serum following intravenous injection. The half-time of the process is 11.0 days.

which time arterial and venous D_2O concentrations are equal. Thus, the first observation can easily be made after the disturbance due to mixing has subsided. The volume of dilution may then be obtained from extrapolation of the later data to zero time, based on the linear slope of the logarithmic plot.

C. Two-Compartment Systems—Steady State and Quasi-steady State

Usually the number of compartments determines the number of variables, and the number of variables determines the minimum number of exponential terms in the solution. However, in closed systems,the equation for conservation of tracer may be applied to reduce the number of variables by one, and thus reduce the number of exponential terms by one. Furthermore, if the system is in the steady state, $\Phi_{12} = \Phi_{21}$. Suppose the tracer is initially injected into Compartment 1.

$$P_{10} = P_1 + P_2 \tag{4}$$

and

$$dP_1/dt = -dP_2/dt = -\Phi_{12}p_1^* + \Phi_{12}p_2^* \tag{5}$$

The use of Eq. (4) permits the elimination of one variable from Eq. (5), so that the solution is characterized by only a single exponential term, as follows :

$$(p_1^*/p_{1\infty}^*) - 1 = (S_2/S_1)e^{-k_{12}[(1+S_1/S_2)]t} \tag{6}$$

As in the one-compartment system, the ratio in p^* may be replaced by a ratio in P or p, without altering the slope of the function or the value of k_{12}. In this case, the slope of the function is not simply related to the half-time of exchange of either S_1 or S_2, which is given by $0.693\ S_i/\Phi_{12}$.

Many examples of systems of this type are known, as for example the diffusion of radioactive water (THO) into beef red cells (Villegas *et al.*, 1958) as shown in Fig. 2. However, even in this case, in which the data fall quite nicely on a straight line when plotted logarithmically according to Eq. (6), one cannot be sure that the system is indeed a two-compartment one. Thus, no data are available about the behavior of the system before the first point, 2 milliseconds after mixing, nor after the last point, 14 milliseconds after mixing. Furthermore, no study has been made of the efflux of THO from labeled cells into unlabeled medium. In this case the exchange takes place so quickly that there is no net water shift between the interior of the cell and the environment (Paganelli and Solomon, 1957), and the steady state is maintained throughout the experiment.

The application of Eq. (6) depends upon a reliable estimate of $p_{1\infty}^*$ and a knowledge of S_1 and S_2. Sometimes these are difficult to obtain, and another method may be used to provide an approximate solution (Solomon, 1952). The exponential term in Eq. (6) is expanded in series, and the resultant equation is divided by t. As t goes to zero,

$$\lim_{t \to 0} \frac{(1 - p_1^*/p_{10}^*)}{t} = k_{12} \tag{7}$$

In studies of the uptake of Na by human red cells a similar equation has been shown to provide values for k_{12} within about 20% of those obtained from the integrated solution (Solomon, 1952).

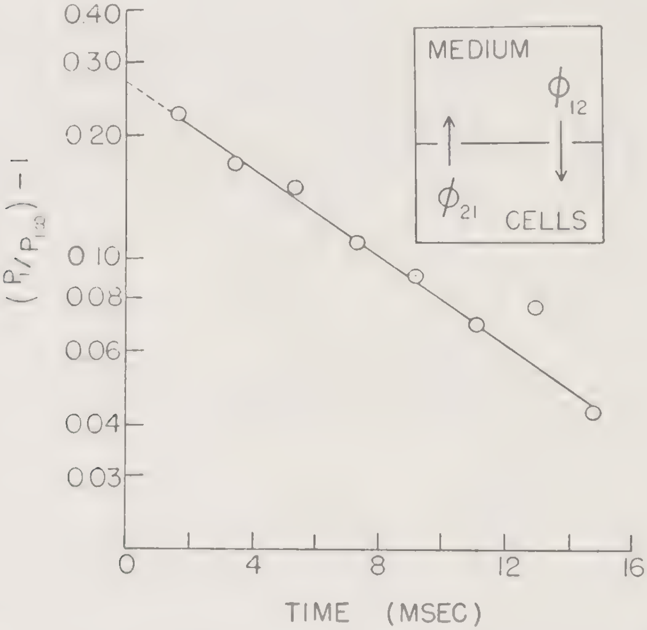

FIG. 2. Diffusion of tritium-labeled water into beef red cells. The rate constant for the reaction is 0.1 msec^{-1}. If the rate of efflux is equal to the rate of influx, half of the water in a tritium-labeled red cell would exchange with the water in an infinite extracellular reservoir in 6.9 msec.

Frequently, the steady state cannot be achieved in experiments with biological systems ; it may be approached approximately, and we are faced with a "quasi-steady state." An example may be found in the uptake of K by human red cells. Although, as will be shown later, this is not a true two-compartment system, the K flux into the cells appears to follow two-compartment kinetics. When incubated *in vitro* in their own plasma, human red cells gain K at a rate of 0.14 meq/liter cell hour (Streeten and Solomon, 1954). This represents a negligible percentage gain in cell K which is about 94 meq/liter cell, and a fairly small percentage loss in plasma K (Compartment 1), amounting to about 3% per hour, or 12% over the usual 4-hour experiment. At first sight, it would appear that such a shift would be negligible in a biological experiment. However, it has been shown that considerable difference exists in the fit of the data to theory, depending on the method used to obtain $p_{1\infty}$. The value of $p_{1\infty}$ may be obtained by use of the initial value of S_1 which is assumed to remain constant throughout the course of the experiment; alternately, the calculation for $p_{1\infty}$ may be corrected for the changes that take place as the plasma loses K to the cells. In the former method, $p_{1\infty}$ is obtained by calculation from the radioactivity added, the initial hematocrit reading, and the initial relative cell and plasma K concentrations, according to the following equation:

$$p_{1\infty} = p_{10}\{1 + [\mathrm{K}]_c H/[\mathrm{K}]_p(1 - H)\}^{-1} \tag{8}$$

in which [K] represents K concentration and the subscripts c and p represent cell and plasma, respectively; H is the hematocrit reading. Both methods of calculation lead to a linear relationship between the ln $[(p_1/p_{1\infty}) - 1)]$ and time, as can be seen from the insert in Fig. 3. It might be concluded that both methods provide an equally good fit to the experimental data, but such a conclusion is not warranted, as a closer examination reveals. For this purpose, the predicted time course of p_1 is compared with that observed experimentally. If S_1, S_2, and $p_{1\infty}$

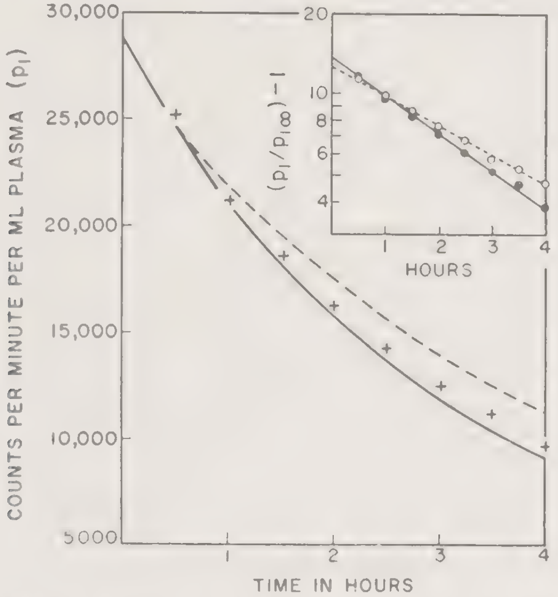

FIG. 3. Loss of K^{42} from plasma in which human red cells are suspended. The crosses represent the experimental observations. The dashed line is the predicted time-course, calculated by adjusting the infinite time value of $p_{1\infty}$ during the course of the experiment. The full curve represents the predicted time-course if $p_{1\infty}$ [calculated from the initial hematocrit and concentrations according to Eq. (8)] is assumed to remain constant over the course of the experiment. The insert shows the time-course of the function $[(p_1/p_{1\infty}) - 1]$ plotted semilogarithmically according to these two methods.

are known, p_1 may be calculated from Eq. (6) together with the value of k_{12} obtained from the slope of the logarithmic line. It can be seen from Fig. 3 that the predicted form of the time-course does not provide a good fit to the data in either case, though the method involving Eq. (8) is to be preferred. We may conclude that the logarithmic method of plotting flatters the data.

The use of the logarithmic scale makes possible the rapid calculation of fluxes from analytical data. However, the fact that the points fall on a straight line, as predicted by Eq. (6), clearly does not provide unequivocal proof that the time-course of the reaction is that predicted by the equation. One reason for this is the distortion on the logarithmic scale; another lies in the process of integration used to solve Eq. (5). Each new determination of plasma radioactivity represents an extension of the limits of an integral, and thus the relative contributions of the individual later determinations are suppressed in the powerful process of integration. If the data are bad enough, this powerful method may indeed be required; however, if the data are good, variations in the temporal behavior of the system may be lost, so that deviations in behavior of the initial time interval from that in subsequent time intervals are completely obscured. As will be seen in some of the later examples, it is always wise to examine the flux as a function of time before integration on the assumption of a time-invariant flux. Furthermore, the time-course, predicted from the rate constants, should always be compared with the actual data on a linear plot.

D. Two-Compartment Systems—One Compartment and Reservoir

When a compartment in the body takes up radioactivity from a large reservoir such as the plasma, we are usually interested in the fluxes into and out of the compartment. Such systems are open, and the equation of conservation is not easy to apply, since tracer is lost from the system. Otherwise, they may be treated by equations analogous to those for closed systems, but unless the reservoir is of constant composition, more than one exponential term is found in the solution. The two most interesting cases are: (1) uptake from a source in which the tracer concentration is kept constant and (2) uptake from a source in which the tracer concentration is some analytical function of time. In the first case, the physiological requirement of maintaining a constant tracer concentration in the plasma is often difficult to meet, but the mathematical treatment of the data is simple. In the second case, the experiments are easy, and the analysis difficult. The equation for the first case, shown in Fig. 4, follows.

$$dP_2/dt = \Phi_{12}p_{10}^* - \Phi_{20}p_2^* \tag{9}$$

where Compartment 1 is the reservoir and the subscript $2o$ refers to flux from Compartment 2 to the outside. In this case, the system is assumed to be in the steady state, and the reservoir is assumed to be so large compared to the compartment that back flux of tracer from the compartment to the reservoir has no effect on the reservoir. With respect to compartment 2, the model cannot discriminate between flux into the reservoir or out of the system, and so the system behaves as if the reactions were irreversible. This is to be recognized as a mathematical artifact, since it is quite possible that the reactions are indeed reversible ; experiments of a different kind would be required to settle this point. The solution to Eq. (9) is

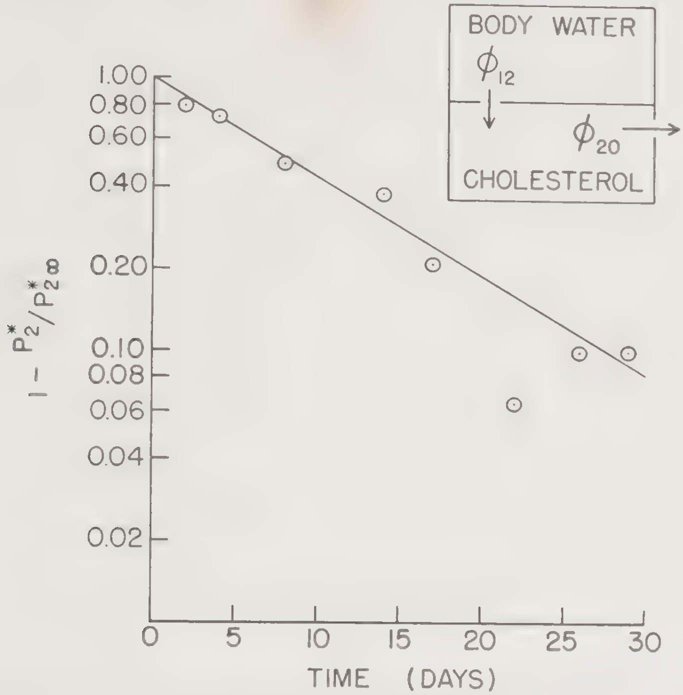

FIG. 4. Uptake of deuterium from body water by cholesterol in man. The deuterium content of the body water is kept approximately constant over the course of the experiment. p_2^* represents atom per cent excess D in cholesterol.

$$p_2^* = p_{10}^* (1 - e^{-k_{2o}t}) \tag{10}$$

and since $p_{10}^* = p_{2\infty}^*$

$$1 - p_2^*/p_{2\infty}^* = e^{-k_{2o}t} \tag{11}$$

An analogous equation applies to the formation of a radioactive isotope by bombardment in a nuclear reactor. The time required to reach equilibrium activity depends only on the rate of decay of the isotope.

while the maximal amount of activity that can be induced depends only on the rate of production of the isotope. Similarly, in a biological system, the time for attainment of $p^*_{2\infty}$ depends only on the rate constant for its destruction k_{20}, and the equilibrium specific activity is determined by p^*_{10}.

An example is afforded by the experiments of London and Rittenberg (1950) on the formation of deuterium-labeled cholesterol in man from a constant pool of deuterium in the body water. Table I shows the data given by the authors, and Fig. 4 shows a semilogarithmic plot of $(1 - p^*_2/p^*_{2\infty})$, as calculated from these observations. Atom per cent excess deuterium may be treated as if it were the specific activity of a radioactive isotope. In view of the considerable variation in the body water deuterium concentration as shown in Table I, the fit seems quite good. London and Rittenberg have calculated that the time required for the function $(1 - p^*_2/p^*_{2\infty})$ to reach half its infinite time value is 8.3 days. Thus k_{20} is 0.083/day (0.693/8.3), and the average life of a cholesterol molecule, assuming that the label stays with the molecule throughout its life, is $1/k_{20} = 12$ days. We have already assumed that Compartment 2 is in the steady state with respect to its cholesterol content, and hence, the rate of formation of cholesterol equals its rate of destruction, so that 8.3 days is also the half-time for synthesis of the cholesterol pool. From Eq. (10), we would expect the atom per cent excess deuterium in the cholesterol to equal that in the body water at infinite time but, as can be seen from Table I, it reaches only about 8% of this expected maximum. Thus, the body water cannot be the direct precursor of all the hydrogen in the cholesterol molecule; instead there

TABLE I

DEUTERIUM CONCENTRATION (ATOM PER CENT EXCESS D) IN BODY WATER AND IN SERUM CHOLESTEROL DIGITONIDE

Days	Body water	Serum cholesterol digitonide	Days	Body water	Serum cholesterol digitonide
0–2	0.48		21–22	0.61	
2		0.011	22		0.051
4		0.015	25	0.51	
4–5	0.70		26		0.049
6–7	0.67		29		0.049
8		0.028	36		0.057
10–11	0.68		37	0.65	
14		0.034	43		0.054
15–16	0.64		44	0.70	
17		0.043	52		0.053

must be another pool containing labeled hydrogen at a lower atom per cent of deuterium. This presumably comes about by dilution of the body water pool with hydrogen obtained from an extraneous source, such as food. Alternatively, some of the hydrogen atoms in cholesterol may exchange very slowly with body water hydrogen. Thus, p_{10}^* as determined from the plasma atom per cent excess D would not necessarily describe the isotopic composition of the precursor compartment. However when $p_{2\infty}^*$ is determined experimentally, no measurement of p_{10}^* is required (beyond the demonstration that it remains constant), and the rate constant k_{20} can be found directly from Eq. (11).

When the tracer concentration in the reservoir compartment is time dependent, the equation is

$$dP_2/dt = \Phi_{12}p_1^* - \Phi_{20}p_2^* \tag{12}$$

If we assume again that the reservoir is so large compared with Compartment 2 that flux in the direction 2 to 1 does not affect p_1^*, the system behaves as if the reactions were irreversible, and Bateman's equations (1910) for the growth and decay of naturally radioactive isotopes may be applied. In these equations a single exponential term is required for each isotope in the chain, just as a single exponential term is required for each compartment. As we have seen, when the equation of tracer conservation can be used to eliminate one variable, the number of exponential terms is reduced by one, so that only a single exponential is sufficient to characterize a closed two-compartment system. A similar reduction occurs when uptake from a constant reservoir is considered. As usual, the rate constants are obtained from the values of the exponents.

Robertson (1957) has pointed out that the solution to Eq. (12), for the steady state and with p_1^* equivalent to any function of time $f(t)$, is given by

$$p_2^* = e^{-k_{20}t}\int f(t)k_{20}e^{k_{20}t}dt + \alpha e^{-k_{20}t} \tag{13}$$

where α is the constant of integration. If $f(t)$ is integrable, p_2^* may be readily determined. For example, $f(t)$ may be given as the sum of two exponentials which often is sufficient to describe the loss of tracer from the plasma in man.

$$f(t) = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t} \tag{14}$$

In Eq. (9), efflux from Compartment 2 was considered to leave the system completely and was described by the symbol, Φ_{20}. If the reservoir is large, the return of the efflux to it will not affect reservoir tracer concentration. In this case an exactly similar equation can be written in which the subscript 21 is substituted for $2o$, and k_{2o} is everywhere replaced with k_{21}. Since the subsequent example will represent such a system, this substitution has been made in the following solution to Eq. (13).

$$p_2^* = \frac{k_{21}C_1}{k_{21}-\lambda_1}e^{-\lambda_1 t} + \frac{k_{21}C_2}{k_{21}-\lambda_2}e^{-\lambda_2 t} + \alpha e^{-k_{21}t} \tag{15}$$

If $p_2^* = 0$ at $t = 0$,

$$\alpha = -\frac{k_{21}C_1}{k_{21}-\lambda_1} - \frac{k_{21}C_2}{k_{21}-\lambda_2} \tag{16}$$

An example will be found in the experiments of Moser and Emerson (1955) on the uptake of P^{32} by phospholipids from plasma phosphate. They have shown that the time-course of urine specific activity, which reflects plasma specific activity, is given by the sum of two exponentials, as in Eq. (14). Figure 5 shows the relative time-course of the specific activity of urine phosphate and plasma phospholipid. Moser and Emerson were unable to extract the values of the three exponents in Eq. (15) from the phosphate and phospholipid curves shown in Fig. 5. Although they found values for λ_1 and λ_2, they could not determine k_{21} by the usual methods of graphical analysis which are discussed in the next section. Curves, such as the phospholipid curve in Fig. 5, which

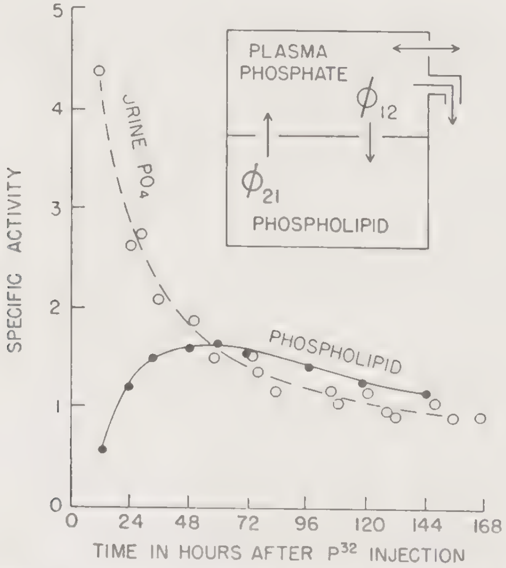

FIG. 5. Uptake of P^{32} by phospholipid from plasma phosphate in man. The dashed curve represents the specific activity of urine phosphate which is considered representative of plasma phosphate (p_1^*). The bottom curve shows the specific activity of phospholipid in the plasma (p_2^*).

are composed of exponentials with both positive and negative coefficients, are particularly difficult to fit by graphical analysis, since the exponentials are often not uniquely determinable. This is particularly true when the curve contains three exponentials as in Eq. (15). Consequently, the authors had recourse to a numerical method. Their heroic procedure involved the solution of Eq. (15) for k_{21} separately at 24, 48, 72, and 96 hours after injection of the tracer. Values of C_1, C_2, λ_1, and λ_2 were obtained from the urine specific activity curve. For any value of t, p_2^* is known from the plasma phospholipid curve, so that only k_{21} remains unknown in Eqs. (15) and (16). Table II gives the results of their calculations which give an over-all mean of 0.014 ±0.0006 hr^{-1} for k_{21}. As the standard error of the mean is 4%, this method leads to a relatively precise determination of the rate constant. Alternatively k_{21} could be determined by a graphical integration of the areas under the curve in Fig. 5 according to the method given by Zilversmit *et al.* (1943).

TABLE II

VALUES OF k_{21} CALCULATED BY NUMERICAL METHOD

	k_{21} in hours^{-1} Calculated from observation times of			
Patient	24 hrs.	48 hrs.	72 hrs.	96 hrs.
P.A.	0.016	0.017	0.015	0.013
O.B.	0.016	0.018	0.019	0.014
A.S.	0.017	0.018	0.014	0.012
C.K.	0.010	0.011	0.011	0.010
R.C.	0.013	0.015	0.013	0.011
F.H.	0.011	0.013	0.012	0.011

Mean ± Standard Error: 0.014±0.0006

E. Graphical Analysis

In the experiments of Moser and Emerson the specific activity of the urine phosphate was found to follow a smooth curve that could be described by the sum of two exponentials, as in Eq. (14). The method by which the values of the coefficient and the exponent for each term may be obtained is illustrated in Fig. 6 and described fully in the caption. This method of graphical analysis may be extended to cases where the curve rises to a maximum and then falls, in which case the exponentials have coefficients of each sign. More complicated curves may require the use of additional exponential terms. The existence of an exponential equation which provides a quantitative description of a curve does not, however, serve as evidence that the curve is representative of a system whose kinetics involves exponentials. Two independent adjustable constants per term provide a tremendous latitude in synthesizing any curve. Independent evidence is necessary in order to relate the synthesized curve to the kinetics of the process. In the example given by Moser and Emerson, the value of Eq. (14) does not lie in

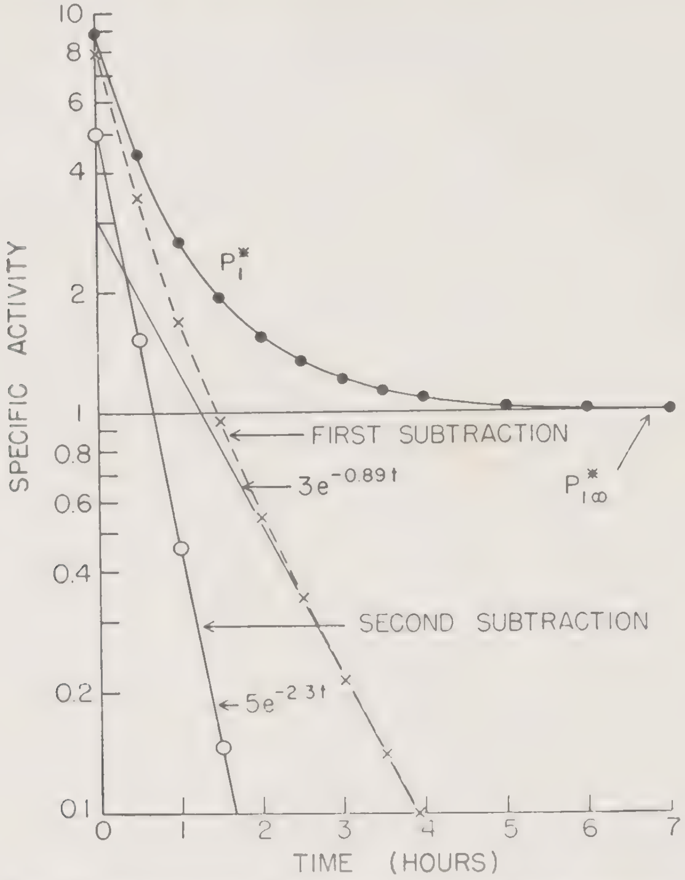

FIG. 6. This figure illustrates the usual method of analyzing a curve graphically. The curve follows the general equation

$$p_1^* = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + p_{1\infty}^*$$

First the value of $p_{1\infty}^*$ is determined, either experimentally or from the asymptote of the curve, as in the present example in which $p_{1\infty}^* = 1.0$. Then $p_{1\infty}^*$ is subtracted from the curve, and the resulting curve is plotted on the same piece of logarithmic graph paper as the first subtraction. Its asymptote is drawn and extended to the origin. The value of the asymptote at zero time is 3.0, which represents C_2. The asymptote falls to a value of 1.5 at 0.78 hours, so its slope is $-0.693/0.78 = -0.89$, and λ_2 is 0.89. The asymptote, whose equation is $3e^{-0.89t}$ is then subtracted from the curve labeled " first subtraction " to yield the " second subtraction ". This is found to be a straight line that follows the equation $5e^{-2.3t}$. Thus, $p_1^* = 5e^{-2.3t} + 3e^{-0.89t} + 1.0$.

the analysis of the plasma PO_4 disappearance; it is valuable as a tool in the determination of the rate constant k_{21}. Graphical analysis of plasma disappearance curves is particularly useful in other instances of this sort.

When the curve can be described by a single exponential term, a straight line is obtained by the conventional methods of graphical representation. Often the method of least squares is used to determine the slope of the line and thus to evaluate the rate constant. However, Roseveare (1931) has pointed out that this method leads to incorrect conclusions because the quantity measured is the specific activity and the quantity plotted is the logarithm of the specific activity. In the specific case in which the error in time measurement is small compared with the error in specific activity (which is considered to be constant in quantity rather than a constant percentage error), the earlier points have a much greater weight than the later points. For example, points obtained when the process is 90% complete should be given only one-hundredth the weight of the initial point. Roseveare gives the necessary equations by which the points may be weighted in order to apply the method of least squares.

It should be pointed out that in many of the equations used in compartmental analysis p^*_{10} or $p^*_{1\infty}$ is used as a constant divisor. When this is the case, it is essential that replicate measurements of these particularly important points be made in order to minimize error.

F. The Precursor Criterion

The experiments of Moser and Emerson also illustrate very nicely the application of the precursor criterion. Zilversmit *et al.* (1943) have established a very useful criterion for determining when one compartment, or substance, is the direct precursor of another. As previously stated, Eq. (12) is valid for a p^*_1 which follows any arbitrary time-course. If the second compartment is in the steady state, so that $\Phi_{12} = \Phi_{20}$, and S_2 is constant,

$$p^*_1 = p^*_2 \quad \text{for} \quad dp^*_2/dt = 0 \tag{17}$$

Since p^*_2 is initially zero, it will reach its maximum value when it equals p^*_1, provided the system is adequately described by Eqs. (12) and (17). In order for these equations to hold, it is necessary that Compartment 1 constitute the only route into Compartment 2. In other words, Compartment 2 must either be a dead-end compartment, or material must leave by an irreversible pathway. Under these conditions, compliance with Eq. (17) indicates that Compartment 1 is the precursor compartment for Compartment 2. Such a situation is illustrated in Fig. 5, where the plasma phosphate (measured as urine phosphate) appears to be the precursor of the liver phospholipid. The presence of small or rapid compartments which do not affect the over-all kinetics of the reaction cannot, of course, be eliminated. To apply the Zilversmit criterion, only the time-course of the specific activity in the two compartments is required and no mathematical solution for p_1^* or p_2^* need be obtained. Thus, this criterion is of very general applicability.

G. Two-Compartment Systems—One Leaky Compartment and an Infinite Reservoir

Keynes and Lewis (1951) have treated a nonsteady-state closed two-compartment system, namely, the exchange of K in resting crab nerve. The total K content in the nerve decreases approximately exponentially, making a negligible contribution to the K content of the large reservoir. Their equations follow.

$$dS_2/dt = \Phi_{12} - \Phi_{21} \tag{18}$$

Since $S_2 = S_{20}e^{-\beta t}$, $dS_2/dt = -S_{20}\beta e^{-\beta t}$. Also $\Phi_{21} = k_{21}S_2$, therefore

$$\Phi_{12} = S_{20}(k_{21} - \beta)e^{-\beta t} \tag{19}$$

Since the reservoir is considered infinite,

$$dP_2/dt = \Phi_{12}p_{10}^* - \Phi_{21}p_2^* \tag{20}$$

which has the solution for the initial condition $P_2 = 0$ at $t = 0$

$$P_2 = S_{20}p_{10}^*(e^{-\beta t} - e^{-k_{21}t}) \tag{21}$$

Figure 7 shows the agreement between the calculated kinetics and the experimental points. In this case, the difference between the two exponentials is more than threefold, so that the two components can easily be separated graphically.

H. Two-Compartment Systems with Invisible Third Compartment

Another example of the application of a graphical method for the solution of a reservoir uptake problem is given in Fig. 8, which shows K^{42} exchange between plasma and brain in a normal adult rat as studied by Katzman and Leiderman (1953). Since the specific activities of plasma and brain K do not come to a common asymptote, it is clear that some factor must modify the tendency to uniform specific activity. This factor will be shown to be a brain compartment of moderate size which exchanges K at a rate slow compared with exchange in the major brain compartment. Under these circumstances, use of the specific

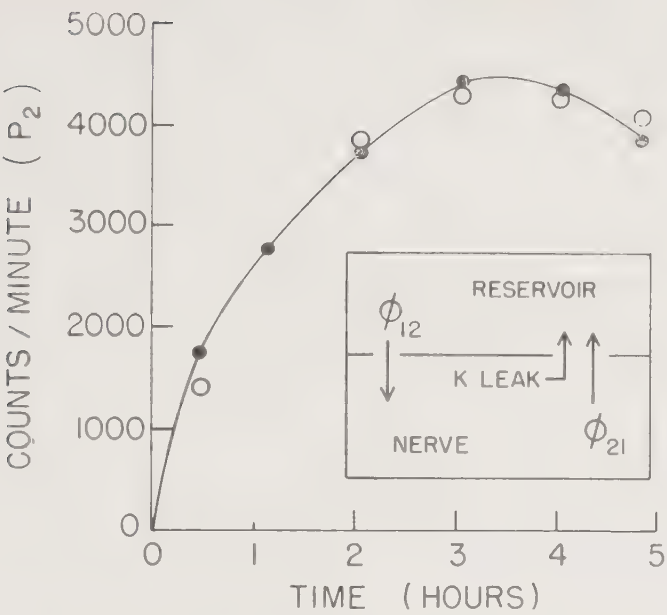

FIG. 7. Uptake of K^{42} by a whole *Carcinus* nerve from an essentially infinite reservoir. The filled circles and the solid line represent the experimental data. The open circles are theoretical values calculated by Keynes and Lewis (1951) according to Eq. (21).

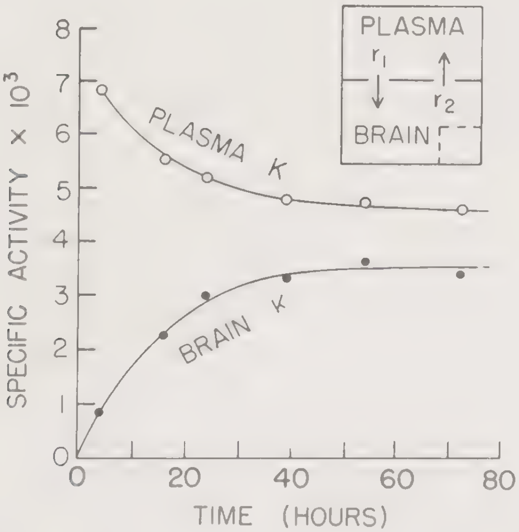

FIG. 8. Uptake of K^{42} by adult rat brain from blood. The two specific activity curves do not come to a common asymptote, indicating that the process is complicated by another compartment.

activity of the whole brain as driving force is not warranted. Instead, the authors give the following equation.

$$dp_2/dt = r_1p_1 - r_2p_2 \tag{22}$$

Since the authors did not measure brain water or plasma water directly, they expressed p_1 in terms of counts per minute per milliliter plasma (cpm/ml plasma) and p_2 in terms of cpm per gm brain. Although the units in which the rates r_1 and r_2 are expressed are unusual, dp_2/dt has units of amount of radioactivity per unit weight of brain and unit time. If the compartments are uniform, and the system is in the steady state, r_1 may be interpreted as the amount of plasma K (expressed as ml plasma) that enters the brain in unit time per gram of brain. r_2 is the fraction of brain K that leaves per unit time.

TABLE III

CALCULATION OF r_1 AND r_2 FOR K^{42} EXCHANGE IN NORMAL ADULT RAT BRAIN

Time hrs	dp_2^*/dt cpm/mole hr	$p_1/[K]_2$ cpm kg/mole liter	p_2^* cpm/mole	Pairs of points hrs		r_1		r_2
4	0.164	0.365	0.85	4 + 16		0.542		0.040
16	0.066	0.289	2.28	4 + 24		0.535		0.037
24	0.058	0.315	3.01	4 + 39		0.530		0.036
39	0.026	0.272	3.32	4 + 54		0.535		0.037
54	0.007	0.264	3.67	4 + 72		0.540		0.038
72	0.003	0.228	3.42	16 + 24		0.430		0.028
				16 + 39		0.475		0.031
				16 + 54		0.495		0.034
				16 + 72		0.495		0.034
				24 + 39		0.515		0.035
				24 + 54		0.565		0.040
				24 + 72		0.530		0.036
				39 + 54		0.595		0.041
				39 + 72		0.565		0.039
				54 + 72		0.535		0.037
					Mean	0.527	Mean	0.036
					Standard Error	0.0095	Standard Error	0.0009

The brain K concentration $[K]_2$ was measured in units of moles per kilogram and found to remain constant throughout the experiment. When Eq. (22) is divided by $[K]_2$, the following equation is obtained.

$$dp_2^*/dt = r_1p_1/[K]_2 - r_2p_2^* \tag{23}$$

To evaluate dp_2^*/dt graphically, a smooth curve is drawn through the experimental points. The slope of this curve is then calculated from tangents drawn by eye. All the other variables in Eq. (23) can be measured directly. The two constants r_1 and r_2 may then be obtained by simultaneous solution of pairs of equations, using all the possible permutations of points as shown in Table III. Although the scatter of the points is relatively large, no trend seems evident, and the standard error of the mean is less than 2.5%.

When r_1 is applied to the plasma K concentration, the influx to the brain is calculated to be 2.9 moles per kilogram brain per hour. Efflux obtained by a similar calculation is 3.6 moles per kilogram brain per hour. Since the system has been shown to be in the steady state with respect to brain K concentration, these fluxes should be equal. Since they are not, some of the K in the brain does not participate as a driving force in the reaction studied. The data show that only about 80% of the brain K exchanges over a 72-hour period.† The shape of the curves in Fig. 8 suggests that the exchange of the remaining K must be appreciably slower than that of the major fraction. Subsequent to the publication of Katzman and Leiderman's paper, the same data were submitted to analysis on the analog computer, and the results were verified exactly.

I. Numerical Methods

The power of a numerical method involving a graphical estimation of the slope of a curve has been illustrated in the preceding section. However, there are many numerical methods that provide more elegant measurements of the slope of a curve than can be obtained by visual estimation. In the author's experience, the easiest of these is the three-point differentiation formula given in Milne (1949) for calculating slopes from evenly spaced points. For three observations, taken at times 0, 1, and 2, with an interval h between each pair of observations, the slopes at times 0, 1, and 2 are

$$(dy/dt)_0 = (1/2h)(-3y_0 + 4y_1 - y_2) \tag{24a}$$

$$(dy/dt)_1 = (1/2h)(-y_0 + y_2) \tag{24b}$$

$$(dy/dt)_2 = (1/2h)(y_0 - 4y_1 + 3y_2) \tag{24c}$$

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† Robertson (1957) has suggested that Katzman and Leiderman's calculations could have been simplified by making the substitution, $r_1p_1/[\mathrm{K}]_2 = r_2p_1^*$. However, this substitution is equivalent to setting $r_1[\mathrm{K}]_{\mathrm{plasma}} = r_2[\mathrm{K}]_{\mathrm{brain}}$ which cannot be done when there is a reservoir of apparently nonexchangeable brain K.

Unless the data are very good, it may be necessary to draw a smooth curve through the experimental points before applying the numerical analysis. This introduces another variable in the system, but often reduces the problem to a manageable form. The final test of the success of any method of data fitting is usually an empirical one. Does the equation fit the curve, and does it conform to a reasonable model? These two requirements are contradictory with respect to the more powerful methods of numerical analysis. These are usually based on expansions in polynomial series, whereas the models customarily used in biological analysis lead to first-order differential equations whose solutions involve exponentials. Thus, it is possible to use numerical methods to obtain information about slopes of particular segments of curves where exponentials and polynomials lead to equivalent results, but it is not desirable to provide empirical solutions to whole time-courses of experimental results by recourse to polynomial equations. In the author's experience, the data available from biological experiments are not yet accurate enough to make desirable the application of the most powerful methods. Common sense is the best guide. However, the use of the three-point formula, even on a smoothed curve, provides a method of estimating derivatives that is relatively free from personal bias.

Numerical methods of solution possess one very considerable advantage over solutions that can be obtained by integration in that they provide a simple method of treating systems not in the steady state. As already discussed, dP_2/dt equals $S_2 dp_2^*/dt$ only if S_2 is a constant; unless S_2 is a simple and known function of time, numerical methods are essential. In general, variations as large as 10% in S_2 are acceptable, but in cases of doubt, decision can be reached by observing how well the predicted behavior of the dependent variable fits the experimental observations. In studies of K uptake by dog erythrocytes, the cells hemolyze, and the extracellular K concentration rises by as much as 15% during the 8 to 16-hour experimental period. Frazier and associates (1954) calculated rate constants on the basis of a graphical method using a visual estimation of the slope of a smoothed curve and compared their results with those obtained from two-compartment analysis, assuming a steady state and applying an equation similar to Eq. (6). They found that the results obtained by the two methods did not differ significantly.

In the author's laboratory the effect of drugs which cause red cells to lose K has been studied recently. For these purposes a numerical treatment has been devised so that drug-treated cells may be compared with normal cells on a quantitative basis. The specific activity of the plasma compartment is determined at hourly intervals, and the arithmetical mean specific activity for each hourly interval is determined. The influx is next calculated from the following equation :

$$\Phi_{12} = -\frac{\Delta P_1}{\Delta t \bar{p}_1^*} = -\frac{\Delta P_1}{\bar{p}_1^*} \quad \text{when } \Delta t = 1 \tag{25}$$

in which $-\Delta P_1$ is the plasma loss of radioactivity over a 1-hour interval (Δt) and $\bar{p}_1^*$ is the mean plasma specific activity over that interval. Comparison with Eq. (5) shows that the assumptions made are that $dP_1/dt = \Delta P_1/\Delta t$ and that $\Phi_{21} p_2^*$ does not contribute to the plasma radioactivity. The first assumption is seen to be reasonable from the time-course of the cell specific activity, under normal steady-state conditions, as illustrated in the upper half of Fig. 9 which gives the results of a

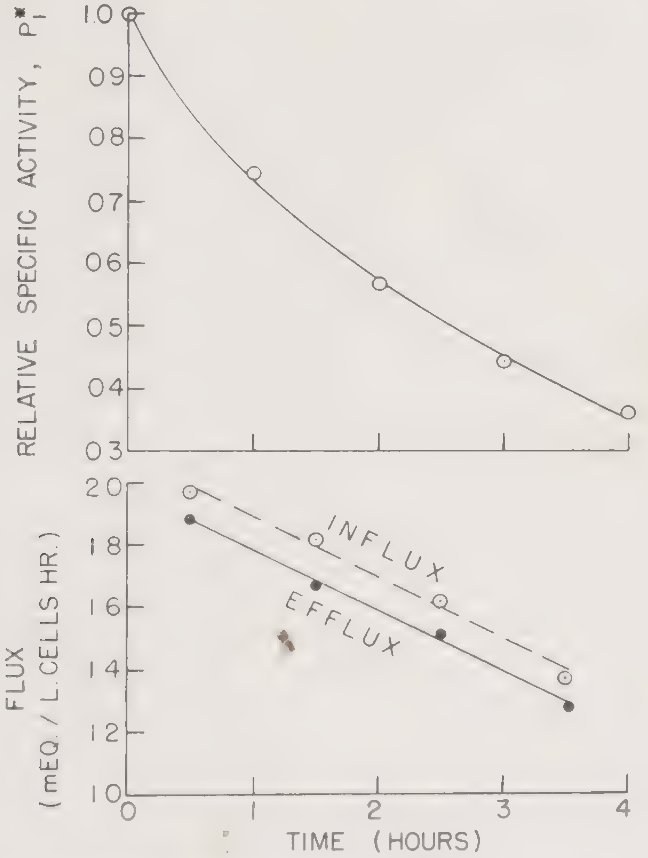

FIG. 9. Graphical method of calculating uptake of K^{42} by human red cells suspended in plasma. The top curve shows the time-course of the fall in plasma specific activity. The bottom figure shows the results of graphical calculations of influx into the cells and efflux from them, carried out as described in the text. Neither influx nor efflux appears to be constant.

typical experiment. Since in these experiments the change in plasma K concentration is slight and the hematocrit reading remains essentially constant, changes in P_1, p_1, and p_1^* follow almost the same time-course. It is relatively difficult to assess the effect of $\Phi_{21}p_2^*$ on plasma radioactivity by analytical means, and an analog computer was used to examine this question. It was found that the contribution from this term was small over the 4-hour period customarily used for experiments of this type. When the influx had been calculated, efflux from the cells was obtained from the difference between net flux and influx. The results of the calculations, shown in the bottom half of Fig. 9, indicate that the flux is certainly not constant when calculated in this manner. Such a trend in fluxes is very different from the constant fluxes required of a true steady-state two-compartment system. Since the fluxes are not time-invariant, Eq. (5) may not be integrated to yield Eq. (6). Nonetheless, when the results are plotted conventionally according to Eq. (6), the usual straight-line relationship is obtained as shown in Fig. 10. On this basis, it might be concluded that Eq. (6) described the system exactly. This false conclusion underlines the weakness of integral solutions in the treatment of biological data.

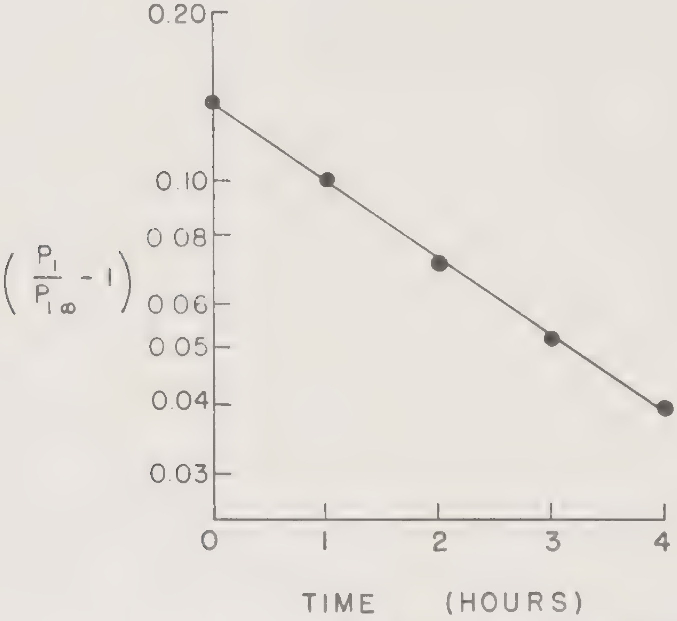

Fig. 10. The experimental data shown in Fig. 9 plotted as a two-compartment system according to Eq. (6).

J. Two-Compartment System—Absorption of Solute and Solvent from Perfused Tube

A more complex two-compartment system is involved in measurement of the absorption of salt and water from the lumen of the intestine as studied by Curran and Solomon (1957). In this system, which is an example of the nonsteady state condition, the lumen is perfused with a solution of known initial composition, and the absorption of salt and water is determined from analysis of the fluid that leaves the perfused segment of the intestine. It is desired to measure the flux of Na across the intestinal mucosa, though the problem is complicated by the simultaneous net movement of NaCl and water out of the perfused segment, as shown schematically in Fig. 11.

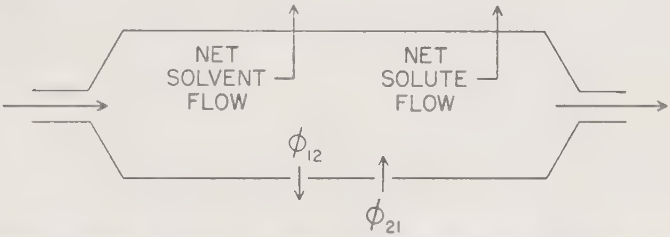

FIG. 11. Schematic diagram showing fluxes in a perfused tube. Both solute and solvent are absorbed from the tube during passage of solution through it.

Under the conditions of the experiment, the external reservoir may be considered as infinite, so there is no back flux of tracer from the blood stream of the animal into the intestinal segment. Then,

$$dP_1/dt = -\Phi_{12}p_1^* \tag{26}$$

Changes in volume have been assumed to be linear with time over the course of the perfusion, so that

$$v_1 = v_{10}(1 - \lambda t) \tag{27}$$

The change in the Na within the lumen is given by

$$dS_1/dt = -\Phi_{12} + \Phi_{21} = -k'_{12}S_1/v_1 + k'_{21}S_2/v_2 \tag{28}$$

in which $k'_{12} = k_{12}v_1$ and $k'_{21} = k_{21}v_2$. These rate constants must be used in this system since v_1 is a variable, and hence k_{12} is not a constant. The fluxes are assumed to be proportional to the sodium concentration, both in the lumen and the plasma, so that k'_{12} and k'_{12} are assumed to be constant. A similar change in rate constants will be required in other systems in which the volume of the compartments does not remain constant.

The solution to these equations is

$$k'_{12} = v_{10}\lambda \left[\frac{\ln(p_1/p_{10})}{\ln(1-\lambda t)} + 1 \right] \tag{29}$$

and

$$\Phi_{21} = (v_{10}\lambda - k'_{12}) \left[\frac{(S_{10}/v_{10})(1-\lambda t)^a - S_1/v_1}{1-(1-\lambda t)^a} \right] \tag{30}$$

in which

$$a = (k'_{12}/v_{10}\lambda) - 1 \tag{31}$$

and

$$\Phi_{12} = k'_{12}(\overline{S_1/v_1}) \tag{32}$$

where $(\overline{S_1/v_1})$ is the time-average concentration of Na in the lumen. It may be obtained either by integrating the luminal Na concentration or, under the conditions of the experiment described, as the arithmetical mean value of the luminal Na concentration. The authors carried out experiments to examine the validity of Eqs. (27) and (29) in the intestine under their conditions. Their results, given in Fig. 12, show that

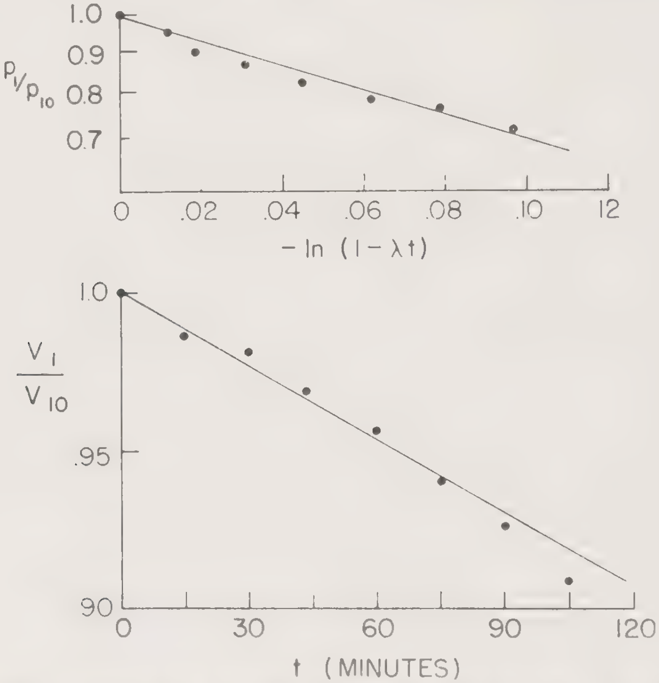

FIG. 12. Uptake of solute and solvent from rat ileum *in vitro*. The top curve, in which p_1/p_{10} is plotted on a logarithmic scale against $-\ln(1-\lambda t)$, shows that Eq. (29) is satisfied. The bottom curve shows that the volume change in the lumen is linear with time as given by Eq. (27).

$\ln p_1/p_{10}$ is a linear function of $\ln(1-\lambda t)$ in accord with Eq. (29), and also validate the linear relationship between r_1 and r_{10}.

III. Three-Compartment Systems

Closed three-compartment systems may all be considered as derivatives of a single general model shown in Fig. 13. The more common arrangements are shown in simpler form in Fig. 14 in which $\Phi_{13} = \Phi_{31} = 0$. These are (1) series, also called a catenary system (more properly catenated) in which tracer is injected into Compartment 1; or (2) parallel, also called mammillary, in which tracer is injected into Compartment 2. The most general system in which all the compartments intercommunicate and no flux equals zero is the one least frequently encountered. The requirements of the steady state impose certain con-

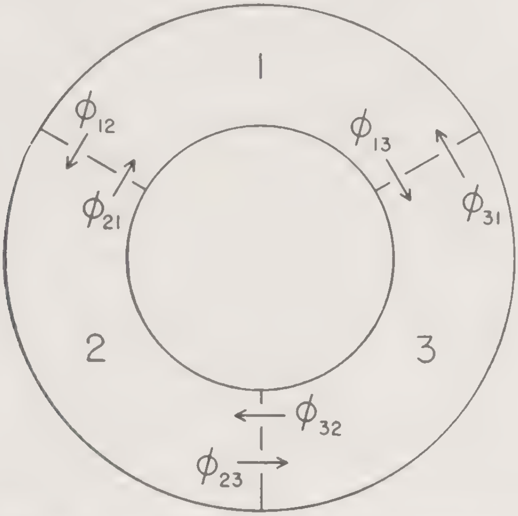

FIG. 13. Schematic drawing showing general three-compartment system.

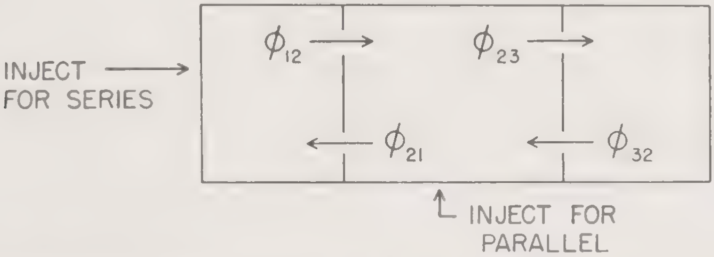

FIG. 14. Schematic drawing showing series and parallel three-compartment models. In the series case, all the activity is placed initially in the first compartment; in the parallel case, it is initially in the center compartment.

ditions on the relations between the fluxes. In both series and parallel cases (Fig. 14), the influx must equal the efflux between each pair of compartments, according to the following equation :

$$\Phi_{12} = \Phi_{21} \; ; \quad \Phi_{23} = \Phi_{32} \tag{33}$$

The conditions of flux equality may be somewhat relaxed in the general case (Fig. 13), for the flux restriction then becomes

$$\Phi_{12} - \Phi_{21} = \Phi_{23} - \Phi_{32} = \Phi_{31} - \Phi_{13} \tag{34}$$

It can be seen that the general system has the capacity to circulate material continuously and still remain in the steady state, thus being more suitable to maintain a continuous flux of material across a cellular membrane, as for example, potassium flux in a human red cell.

General equations covering several varieties of three-compartment systems have been derived by Robertson and associates (1957) and Berman and Schoenfeld (1956), among others. In addition, general treatments such as the elegant ones of Sheppard (1948) and Sheppard and Householder (1951) are applicable.

A. Closed Three-Compartment Systems—Series and Parallel

The following treatment of closed series and parallel cases is that of Robertson *et al.* (1957). Let us assume that the fluxes are related as in Eq. (33) (and make use of the definition $k_{ij} = \Phi_{ij}/S_i$). Then the same set of equations may be used to define both series and parallel cases, and the distinction between them will depend on whether the tracer is initially injected into the center or the end compartment.

$$dp_1^*/dt = -k_{12}p_1^* + k_{12}p_2^* \tag{35a}$$

$$dp_2^*/dt = k_{21}p_1^* - (k_{21} + k_{23})p_2^* + k_{23}p_3^* \tag{35b}$$

$$dp_3^*/dt = k_{32}p_2^* - k_{32}p_3^* \tag{35c}$$

The solution is of the general form given below :

$$p_1^* = C_{11}e^{-\lambda_1 t} + C_{12}e^{-\lambda_2 t} + p_1^* \tag{36a}$$

$$p_2^* = C_{21}e^{-\lambda_1 t} + C_{22}e^{-\lambda_2 t} + p_{1\infty}^* \tag{36b}$$

$$p_3^* = C_{31}e^{-\lambda_1 t} + C_{32}e^{-\lambda_2 t} + p_{1\infty}^* \tag{36c}$$

In the series case, it is assumed that all the tracer is initially in Compartment 1 and that the investigator has access to this compartment ; from this he can determine the values of C_{11}, C_{12}, λ_1, λ_2, and $p_{1\infty}^*$. In the parallel case, it is assumed that the equation for p_2^* is known and all the tracer is initially in Compartment 2. The rate constants for both cases have been worked out explicitly by Robertson *et al.* (1957) and are presented in Parts 1 and 2 of Appendix 1. It can be seen that each k contains contributions from each λ so that no single segment of the curve can be identified with a single compartment. This provides a marked contrast to compartments linked by irreversible reactions in which the k's may be identified with the λ's. The coefficients may also not be identified uniquely with any single compartment, as pointed out in Appendix 2 by A. F. Huxley. This Appendix provides a detailed treatment of the efflux of a labeled ion from a piece of tissue (including intra- and extracellular compartments) into a large external bathing medium.

If it is possible to obtain access to all the compartments during the course of the experiment, the rate constants may be calculated directly from the time-course of radioactivity in each compartment. The equations by which these calculations can be made are given by Hart (1955).

B. Open Three-Compartment Systems—Series and Parallel

Sometimes it is not possible to determine whether a system of compartments is in a series or in parallel. An example is provided in the studies of Gold and Solomon (1955) on Na uptake by human red cells *in vivo*, an open three-compartment system. These authors were interested in establishing the nature of a slow compartment, which became evident about 10 hours after the start of *in vitro* experiments, and was too slow to be studied by the usual *in vitro* methods. Tracer was injected into human volunteers and the relative specific activity of cells and plasma was measured for a 30-hour period. Figure 15 shows the results obtained. The equation governing Na disappearance from the plasma is

$$p_1^* = C_{11}e^{-\lambda_1 t} + C_{12}e^{-\lambda_2 t} \tag{37}$$

When all the activity is initially in the plasma, and Eq. (37) obtains, it is possible to calculate the expected fluxes for parallel and series compartments. In the series case ($\Phi_{13} = \Phi_{31} = 0$),

$$dP_2/dt = \Phi_{21}(p_1^* - p_2^*) - \Phi_{32}(p_2^* - p_3^*) \tag{38a}$$

$$dP_3/dt = \Phi_{32}(p_2^* - p_3^*) \tag{38b}$$

which have the solutions (for $p_2^* = p_3^* = 0$ at $t = 0$)

$$\begin{aligned} p_2^* = B_1e^{\lambda_3 t} + B_2e^{\lambda_4 t} &+ [k_{21}C_{11}(k_{32} - \lambda_1)/\lambda_1']e^{-\lambda_1 t} \\ &+ [k_{21}C_{12}(k_{32} - \lambda_2)/\lambda_2']e^{-\lambda_2 t} \end{aligned} \tag{39a}$$

$$p_3^* = C_1e^{\lambda_3 t} + C_2e^{\lambda_4 t} + (k_{21}k_{32}C_{11}/\lambda_1')e^{-\lambda_1 t} + (k_{21}k_{32}C_{12}/\lambda_2')e^{-\lambda_2 t} \tag{39b}$$

The values for B_1, B_2, λ_3, λ_4, λ_1', λ_2', C_1 and C_2 will be found in Part 3 of Appendix 1.

In this example we do not have access to any single compartment within the cell, but only to the whole cell. Its mean specific activity may be denoted by m_{12}^*, which is given by

$$m_{12}^* = (S_2 p_2^* + S_3 p_3^*)/(S_2 + S_3) \tag{40}$$

In view of the limited access to the separate cellular compartments, S_2 and S_3 cannot be determined separately, although their sum is fixed. Consequently, they are treated as variables and, together with the fluxes, are fitted by eye to provide the best fit to the experimental data, yielding the cell curve shown in Fig. 15. The displacement of the points from the curve before 9 hours is a reflection of the very high initial specific activities in the first few minutes following the injection of radiosodium. This initial fast phase has been neglected in Eq. (37) since interest centers on the period from 10 to 30 hours. Once the fluxes and individual compartment sizes have been found, it is possible to predict

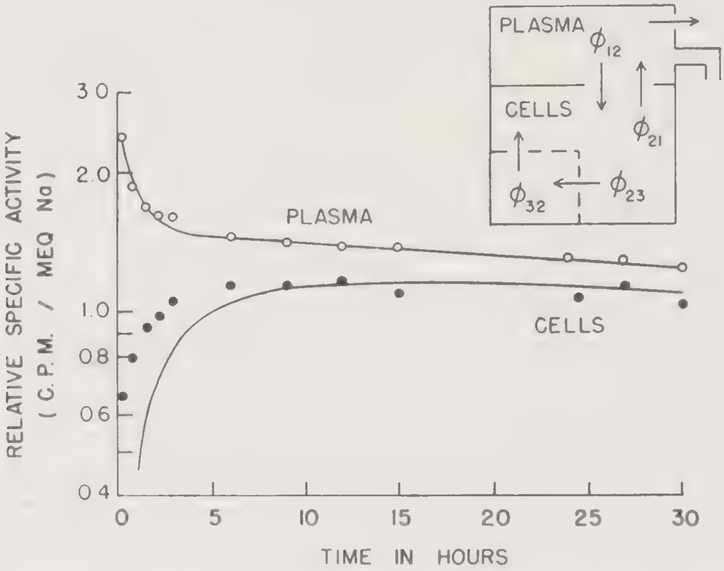

FIG. 15. Uptake of Na^{24} by human red cells from plasma *in vivo*. The two relative specific activity curves, plotted on a common scale, do not come to a common asymptote, indicating that an additional compartment is required to describe the process. The displacement of the points on the cell curve before 9 hours is attributed to the high initial specific activity in the plasma which is 5–10 times greater than the initial plasma specific activity shown. This fast initial phase is not taken into account either in Eq. (37) or in Eqs. (39) from which the cell curve was drawn, since interest centers on the period from 10 to 30 hours.

the time-course of the specific activity in all the compartments using Eqs. (37) and (39). The result of these predictions is shown in Fig. 16.

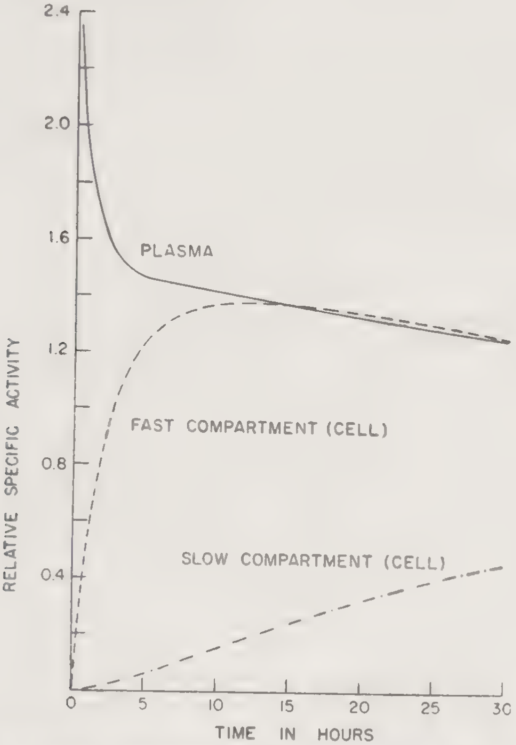

FIG. 16. Schematic drawing showing the predicted relative specific activities in the three compartments of a series model based on constants derived from measurements on the uptake of Na^{24} by human red cells suspended in plasma.

The equations for parallel compartments are derived according to an arrangement of compartments shown in Fig. 13, with $\Phi_{23} = \Phi_{32} = 0$. This system is to be preferred to the parallel case shown in Fig. 14, since in the present case the tracer is initially injected into Compartment 1.

$$dP_2/dt = \Phi_{21}(p_1^* - p_2^*) \tag{41a}$$

$$dP_3/dt = \Phi_{31}(p_1^* - p_3^*) \tag{41b}$$

which have the solutions (for $p_2^* = p_3^* = 0$ at $t = 0$)

$$p_2^* = D_1 e^{-k_{21}t} + [C_{11}k_{21}/(k_{21} - \lambda_1)]e^{-\lambda_1 t} + [C_{12}k_{21}/(k_{21} - \lambda_2)]e^{-\lambda_2 t} \tag{42a}$$

$$p_3^* = D_2 e^{-k_{31}t} + [C_{11}k_{31}/(k_{31} - \lambda_1)]e^{-\lambda_1 t} + [C_{12}k_{31}/(k_{31} - \lambda_2)]e^{-\lambda_2 t} \tag{42b}$$

The values for D_1 and D_2 will be found in part 4 of Appendix 1. An equally good fit to the data has been obtained in the parallel case. Long-term experiments to study the efflux from cells previously incubated with Na^{24} for varying periods might make it possible to discriminate between the series and the parallel arrangements.

C. Closed Three-Compartment Ring System—Use of Analog Computer

The last three-compartment system that will be discussed is the ring arrangement in which each compartment communicates with the other two, as shown in Fig. 13. This system will serve as an introduction to the use of the analog computer in compartmental analysis. The defining equations are

$$dP_1/dt = -\Phi_{12}p_1^* - \Phi_{13}p_1^* + \Phi_{21}p_2^* + \Phi_{31}p_3^* \tag{43a}$$

$$dP_2/dt = \Phi_{12}p_1^* - \Phi_{21}p_2^* - \Phi_{23}p_2^* + \Phi_{32}p_3^* \tag{43b}$$

$$dP_3/dt = \Phi_{13}p_1^* + \Phi_{23}p_2^* - \Phi_{31}p_3^* - \Phi_{32}p_3^* \tag{43c}$$

We have already considered the influx of K into human red cells, which imitates the behavior of a two-compartment system. This is no longer the case when the efflux of radioactivity from previously labeled cells is studied in the steady state (Solomon and Gold, 1955), as can be seen from the time-course of plasma radioactivity shown in Fig. 17. Such a curve cannot be obtained from an equation with only a single exponential, and consequently, the system has been treated as a closed three-compartment system with two of the compartments contained in the cellular phase. The total K content of the two intracellular compart-

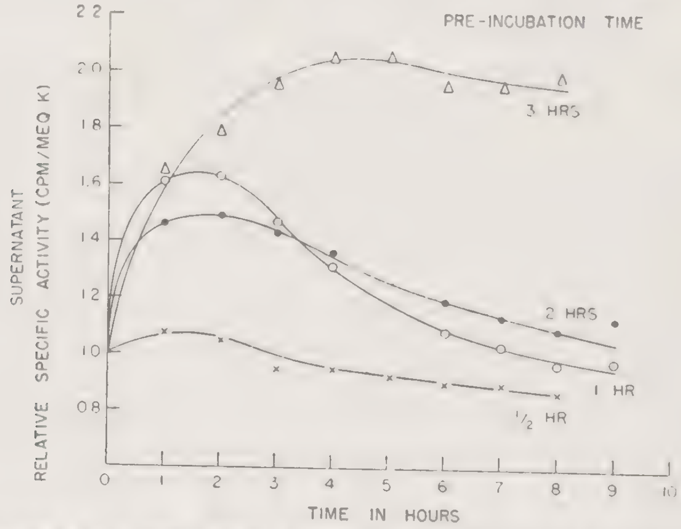

FIG. 17. Efflux of K^{42} from labeled human red cells suspended in plasma. The time of pre-incubation in a radioactive medium is specified for each curve. The relative specific activity of the suspension medium is shown on a time scale which begins when the radioactive cells are resuspended in a nonradioactive medium. The curves are drawn by eye.

ments is known, but its distribution between them is not. As suggested by Solomon *et al.* (1956), the third compartment may reflect an intracellular region that is different from the rest of the cell, or it may reflect an inhomogeneity of the cellular population.

Equations (43) have also been used to assay the effect of ouabain, a drug which inhibits the uptake of K from the medium in which the red cells are suspended. Under the influence of the drug, there is a large increase in the K concentration in the medium (Compartment 1) which may double over the 4–5 hour period of the experiment. This means that S_1 can no longer be treated as a constant, and the system is no longer in the steady state. Since the system is closed, a change in S_1 must be reflected in the sum of S_2 and S_3. However, the change in the sum is proportionately much less since the cells contain perhaps 30 times more K than the medium, and the authors (Solomon *et al.*, 1956) have not taken explicit account of it. They have observed that the rise of K in the medium is linear, so that $S_1 = S_{10}(1 + ct)$ where c is a constant. Substitution of this relationship in the equation for P_1 in Eqs. (43) leads to

$$S_{10}(1 + ct)(dp_1^*/dt) = -\Phi_{12}p_1^* - \Phi_{13}p_1^* + \Phi_{21}p_2^* + \Phi_{31}p_3^* - S_{10}cp_1^* \qquad (44)$$

In order to obtain the rate constants and the fluxes in Eqs. (43) and (44), the set of three equations (43a, b, and c; or 44, 43b and c) is solved on the analog computer. To illustrate the procedure followed, the process will be described in two steps. First it will be assumed that S_1 may be treated as a constant, and the process will be described in detail; then the modifications due to the variation in S_1 will be discussed.

A direct analog computer forms a direct analog to the three compartments assumed to be important in the process studied. The total K content of each compartment is represented by the capacity of an adjustable capacitance, and the flux between compartments is represented by variable resistances. The initial introduction of K^{42} is simulated by putting a charge on one or more of the capacitances; the subsequent history of the system is followed by observation of the potential of each of the three capacitances. Indirect analog computers work on the same general principle as the direct ones.

In compartmental analysis, the unknown is usually the flux between the compartments, the size of the compartment and the equations for the system being known. The proper connections are made on the computer so that the analog represents the equations being studied, and the known values are inserted into the system wherever possible. Then the unknown variable or variables are systematically adjusted until the time-course of the solution given by the analog is as close as possible to the experimental time-course. It is always advisable to study the biological system under as many different conditions as possible, so that the range of possible solutions from the computer is constrained. Also it is advisable to reduce the number of unknowns to the absolute minimum.

Figure 18 is a picture of the screen of a cathode ray tube showing the computer solution for a typical influx experiment into red cells. Figure 19 (bottom curve) shows the relationship between this computer solution and the experimental data. Using the same model, an equally good fit has been obtained in an efflux experiment ; it is illustrated in Fig. 20.

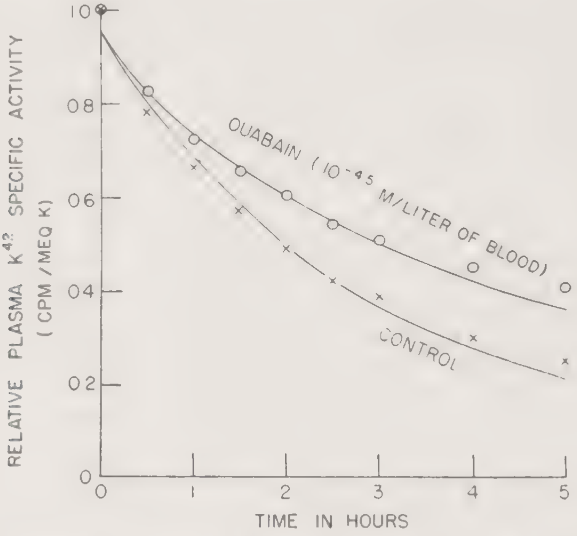

FIG. 19. Efflux of K^{42} from plasma in which human red cells are suspended *in vitro*. The crosses and circles represent experimental data, and the curves are obtained from photographs of the oscilloscope screen of the analog computer.

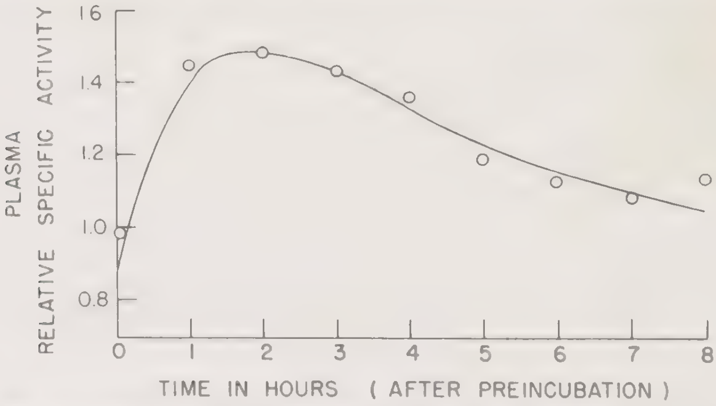

FIG. 20. Efflux of K^{42} from human red cells suspended in nonradioactive plasma. The cells were pre-incubated with K^{42} for a 2-hour period. The open circles represent the experimental data, and the curve has been obtained from a photograph of the oscilloscope screen of the analog computer.

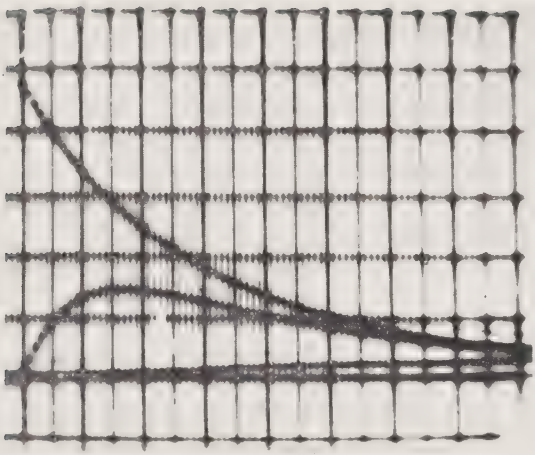

FIG. 18. Photograph taken of the screen of the analog computer cathode ray tube. The three curves represent the relative specific activities in the three compartments; the top curve is the plasma, and the bottom two represent intracellular specific activities. The scale for the ordinates is arbitrary, whereas the abscissas show time in half-hours over an 8-hour period.

When examined in this detail, the agreement between computer predictions and experimental data seems impressive. However, it must be remembered that this agreement depends, in the final analysis, on the judgment of the man who does the fitting. At first sight the use of the computer seems to free the scientist from arbitrary decisions regarding the application of certain models to biological situations, but the freedom is illusory because the data are rarely so good that the final decision is unequivocal. It is this dilemma that makes the use of a computer so difficult since each experiment must be fitted individually to its own set of constants, and the relative goodness of fit is not open to quantitative expression.

In some cases the computer provides a solution to an otherwise insoluble problem. For example, the author has not been able to calculate the rate constants from the analytical solution to the three-compartment system treated above when the relative amount of K in Compartments 2 and 3 is unknown and the relative amount of K^{42} in these compartments at the outset of the experiment is also unknown. The situation is even more complicated when the K content of the plasma changes markedly during the experiment, as is the case for ouabain-poisoned cells. Under these conditions, Eq. (44) is used in place of Eq. (43a). This requires the use of additional computer components, but may be treated readily. The upper curve in Fig. 19 represents a comparison of the computer curve with the experimental data. Even with the additional refinement of compensating for the change in the K concentration in the medium, the computer model presents only an approximation of the true situation, as can be seen from the displacement of the computer curve from the 4- and 5-hour points.†

The analog computer is a particularly useful tool in the calculation of fluxes in open systems, such as uptake from the plasma. In this case it is possible to simulate the time-course of plasma radioactivity directly on the screen of the cathode ray tube and to use this simulated time-course as the input function for the second compartment. This avoids the difficulties in graphical or numerical separation of exponentials in a solution which may contain three or more exponential terms. Brownell and associates (1953) have built an analog computer expressly for compartmental analysis of biological systems and have described its use for the solution of problems of this type. Another feature, the rapidity with which alternative models may be explored, adds considerably to the usefulness of an analog computer, which appears to be a flexible and important tool in studies of compartmental analysis.

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† No account has been taken of the kinetics of the action of ouabain itself on the cell. Since this reaction is almost certainly not instantaneous, it is unlikely that the fit of the computer-generated curve would be good.

IV. Multi-compartmental Systems

In general, compartmental analysis is much more difficult with systems containing more than three compartments. It is no longer practicable to attempt to solve the equations by substitution and integration methods, and the determinant method becomes essential. It is much more difficult to obtain the rate constants and the fluxes explicitly. The questions that have previously been raised about the biological identity of the separate compartments return with increased insistence as the number of compartments increases. It is more than ever necessary to identify each compartment separately, and to isolate one from the other in order to assign the right fluxes to the right barrier. The amount of information necessary for a complete treatment increases many fold. Sheppard (1948) and Sheppard and Householder (1951) have shown that a complete solution for a system of n compartments requires access to each compartment, and $n-1$ tracers. Hart (1955, 1957) has shown that only a single tracer is required if there is access to all the compartments all the time. Robertson (1957) has pointed out that the apparent discrepancy between these requirements arises from the fact that in the first treatment, the specific activities in all compartments and the rate of change of specific activity in one compartment are assumed to be known at one instant of time only; whereas in the second treatment, the entire time-course of the specific activity in each compartment is assumed to be known. These requirements may be contrasted with those for three-compartment systems, in which, as has been shown, access to only a single compartment and the use of only a single tracer will in many cases provide definitive information about the system.

One example will be given of a four-compartment system in which the compartments have been separately identified. Analysis such as this is much simpler in giant-celled organisms than in experimental animals, and simpler in experimental animals than in man. In *Nitella axillaris*, a giant-celled fresh water alga which is shown schematically in Fig. 21, Diamond and Solomon (1959) have identified each compartment and shown that the three intracellular compartments are arranged in series. The time-course of the radioactivity of the cell is shown in Fig. 22. In this case, mathematical analysis has not been necessary to establish the identity of the various compartments since the apparent half-times for exchange of Compartments 2, 3, and 4 are in the ratio of 94,000 : 780 : 1. The procedure for the identification of the compartments follows.

Compartment 2. This compartment was identified as the cell wall. When isolated in contact with a normal solution containing K^{42}, the wall contained 7200 ± 3300 pmoles† K/cm². Under the same conditions, the size of the fastest compartment was 6500 ± 3500 pmoles K/cm², a value similar to that of the isolated cell wall. Next, the K content in the wall was varied by changing the K concentration in the medium over a 10,000-fold range. In these experiments measurements of K content in isolated walls continued to agree with estimates of compartment size derived from experiments with whole cells, as shown in Fig. 23.

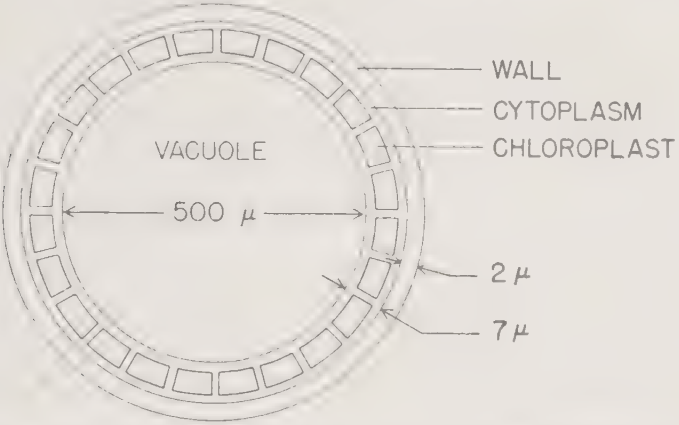

FIG. 21. Schematic drawing showing cell compartments and dimensions in a cross-section of the giant-celled fresh water alga, *Nitella axillaris*.

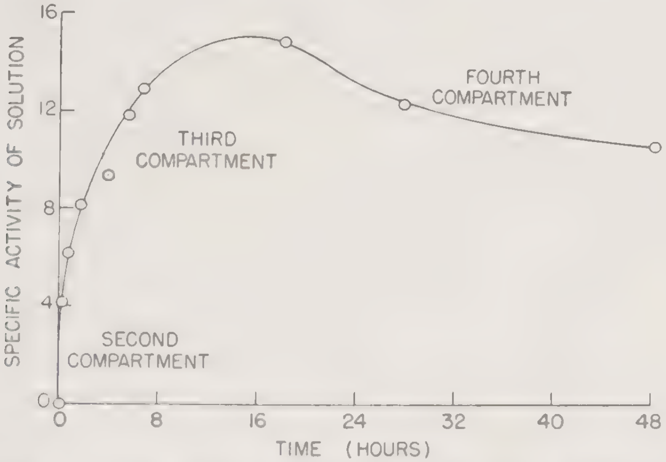

FIG. 22. Loss of K^{42} from labeled *Nitella* into nonradioactive medium. As discussed in the text, the fastest (second) compartment represents the cell wall, the next (third) compartment represents the chloroplast-cytoplasm layer, and the slowest (fourth) compartment represents the vacuole.

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† 1 pmole = 10^{-12} mole

Compartment 4. This, the slowest compartment, was identified as the vacuole. The K content of the vacuole is 1.3 μmoles/cm^2, in good agreement with the figure of 1.2 μmoles/cm^2, the K content of the slowest compartment. The rate of entrance of K into the vacuolar sap is 680 ±40 pmoles/hr cm^2, determined from influx experiments in which samples of sap were isolated and measured, as shown in Fig. 24. From the K^{42} uptake by whole cells after one day, it can be estimated that entry into the slowest compartment proceeds at a rate of 780 ± 150 pmoles/hr. cm^2, a value in good agreement with the direct measurement. It will be noted from Fig. 24 that the time course of K^{42} entrance into

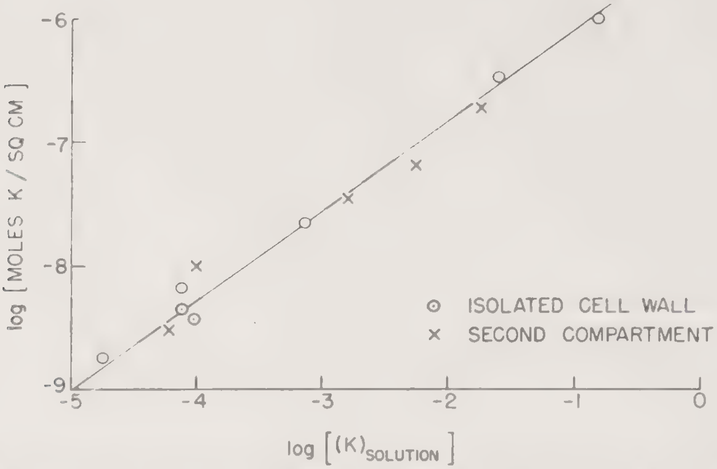


FIG. 23. Relationship between the K content of cell wall and of the fastest intracellular compartment [Moles K/cm^2], as a function of the K concentration of the medium [Moles K/liter]. The circles represent measurements of the isolated cell wall, and the crosses show uptake into the fastest compartment of the intact cell.

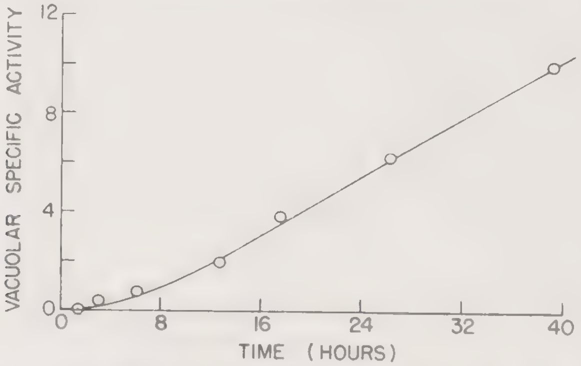


FIG. 24. Uptake of K^{42} in vacuole of *Nitella*. After incubation, the vacuolar contents have been removed from the cell, and the specific activity determined directly.

the vacuole appears to have zero slope at zero time. This relationship is essential for a compartment connected to the reservoir through a compartment whose initial radioactivity content is zero. Since on this time scale, the effect of the fast compartment is unimportant, the zero slope is in agreement with the assignment of the vacuole as the last compartment in the series.

Compartment 3. This compartment has been identified as the cytoplasm-chloroplast layer. Cells could be prepared with practically all of their radioactivity in the medium-speed compartment by incubating them in the usual medium for 30 minutes and rinsing them for 5 minutes. This short time of incubation did not allow an appreciable amount of radioactivity to enter the vacuole, and the rinse removed the activity from the cell wall. The cytoplasm-chloroplast layer could only be imperfectly separated from the other cellular components ; upon isolation it contained 62% of the total cell radioactivity. Nine per cent of the activity was found in the vacuole, and the remaining 29% of the radioactivity was assumed to remain with the cytoplasm which stuck to the cell wall. In view of the incomplete recovery of this fraction, the identification is not as complete as in the case of the other intracellular compartments. However, the major part of the radioactivity is found where predicted by the kinetics of the system; this constitutes a strong argument for the proposed identification.

This compartment may not be considered structurally homogeneous, since it contains chloroplasts which are physically separable from the cytoplasm. Consequently, experiments were carried out to measure the permeability of isolated chloroplasts, resuspended in cell sap. It was found that the K^{42} uptake of chloroplasts had reached its maximum value at the end of 6 minutes, and retained this value over incubation periods as long as one day. Thus this compartment presumably reaches a rapid equilibrium with the cytoplasm, and hence cannot be identified separately since it lies wholly within a slower compartment. This finding illustrates the masking effect which a slow compartment may exercise on a faster compartment which is further along the reaction chain.

V. Summary and Conclusions

Compartmental analysis is seen to provide a powerful tool for the interpretation of tracer experiments. It involves the synthesis of an abstract model system on the basis of which experimental results can be described in a quantitative manner. The mathematical analysis involved in the construction of the model has been examined in some detail. It is seen that the process does not introduce unusual mathematical problems, although the solutions to some of the equations involve many cumbersome manipulations. In the multicompartmental systems, more elegant mathematical methods are required. However, in these cases, the analog computer provides an important and basic tool which allows exhaustive consideration of many alternate models in a rapid and decisive fashion.

The major problem is conceptual. The creation of the model should be followed by further experiments designed to test the validity of its predictions. This involves a rigorous examination in terms of the assumptions inherent in the method of analysis. Searching tests must be applied to ensure that the model conforms to the biological system, both in the number of compartments and in the time invariance of the fluxes in the steady state. When these conditions are satisfied, the information that can be extracted by compartmental analysis provides a detailed and accurate description of important biological parameters.

Appendix I

1. The rate constants for the solutions to Eqs. (35) are given below for the series case ($p_2^* = p_3^* = 0$ at $t = 0$).

$$k_{12} = \frac{C_{11}\lambda_1 + C_{12}\lambda_2}{p_{10}^*} \tag{A 1}$$

$$k_{21} = \frac{k_{12}(\lambda_1 + \lambda_2 - k_{12}) - \lambda_1\lambda_2[1 - (p_{1\infty}^*/p_{10}^*)]}{k_{12}} \tag{A 2}$$

$$k_{32} = \frac{p_{1\infty}^*\lambda_1\lambda_2}{p_{10}^* k_{21}} \tag{A 3}$$

$$k_{23} = \frac{\lambda_1\lambda_2[1 - (p_{1\infty}^*/p_{10}^*)]}{k_{12}} - k_{32} \tag{A 4}$$

2. The rate constants for the solutions to Eqs. (35) are given below for the parallel case ($p_1^* = p_3^* = 0$ at $t = 0$).

$$k_{12} = \frac{-[C_{21}\lambda_1 + C_{22}\lambda_2 - p_{20}^*(\lambda_1 + \lambda_2)]}{2p_{20}^*} - \frac{\sqrt{[C_{21}\lambda_1 + C_{22}\lambda_2 - p_{20}^*(\lambda_1 + \lambda_2)]^2 - 4p_{20}^* p_{1\infty}^* \lambda_1\lambda_2}}{2p_{20}^*} \tag{A 5}$$

$$k_{32} = \frac{p_{1\infty}^*\lambda_1\lambda_2}{p_{20}^* k_{12}} \tag{A 6}$$

$$k_{21} = \frac{k_{12}(\lambda_1 + \lambda_2 - k_{12} - k_{32}) - \lambda_1\lambda_2 + k_{12}k_{32}}{k_{12} - k_{32}} \tag{A 7}$$

$$k_{23} = \frac{C_{21}\lambda_1 + C_{22}\lambda_2}{p_{20}^*} - k_{21} \tag{A 8}$$

3. The constants in Eqs. (39) are given below.

Let
$$v = S_3/S_2 \tag{A 9}$$

and
$$j = (k_{21} + k_{32} + vk_{32}) \tag{A 10}$$

Then
$$\lambda_3 = \tfrac{1}{2}[-j - \sqrt{j^2 - 4k_{21}k_{32}}] \tag{A 11}$$

$$\lambda_4 = \tfrac{1}{2}[-j + \sqrt{j^2 - 4k_{21}k_{32}}] \tag{A 12}$$

$$\lambda_1' = \lambda_1^2 - j\lambda_1 + k_{21}k_{32} \tag{A 13}$$

$$\lambda_2' = \lambda_2^2 - j\lambda_2 + k_{21}k_{32} \tag{A 14}$$

$$B_1 = [k_{21}(C_{11} + C_{12}) + (k_{32} - \lambda_1)(\lambda_4 + \lambda_1)k_{21}C_{11}/\lambda_1' + (k_{32} - \lambda_2)(\lambda_4 + \lambda_2)k_{21}C_{12}/\lambda_2']/(\lambda_3 - \lambda_4) \tag{A 15}$$

$$B_2 = -B_1 - (k_{32} - \lambda_1)k_{21}C_{11}/\lambda_1' - (k_{32} - \lambda_2)k_{21}C_{12}/\lambda_2' \tag{A 16}$$

$$C_1 = [(\lambda_4 + \lambda_1)k_{21}k_{32}C_{11}/\lambda_1' + (\lambda_4 + \lambda_2)k_{21}k_{32}C_{12}/\lambda'_2]/(\lambda_3 - \lambda_4) \tag{A 17}$$

$$C_2 = -C_1 - k_{21}k_{32}C_{11}/\lambda_1' - k_{21}k_{32}C_{12}/\lambda_2' \tag{A 18}$$

4. The constants in Eqs. (42) are

$$D_1 = -C_{11}k_{21}/(k_{21} - \lambda_1) - C_{12}k_{21}/(k_{21} - \lambda_2) \tag{A 19}$$

$$D_2 = -C_{11}k_{31}/(k_{31} - \lambda_1) - C_{12}k_{31}/(k_{31} - \lambda_2) \tag{A 20}$$

Appendix 2

(by A. F. Huxley)

The exchange of a labeled ion between a bathing solution and a piece of tissue (e.g., a nerve or a muscle) is often dealt with on the assumption that the system consists of three compartments in series, the compartments being (*1*) bathing solution, (*2*) extracellular fluid in the tissue, (*3*) the cells themselves. The exchange is commonly found to consist of a fast and a slow phase, which correspond roughly (but not exactly) with the exchange of extracellular and intracellular material, respectively. It is widely recognized that (as pointed out in Section III, A) the half-times of the two components are not exactly equal to what would be given respectively by (*a*) exchange between bathing fluid and extracellular space, the cell membranes being impermeable and (*b*) exchange between bathing fluid and cells, the extracellular space being always in equilibrium with bathing fluid. On the other hand, it seems to be commonly assumed that the *sizes* of the fast and slow components correspond exactly to the amounts of the ion in the extracellular space and inside the cells, respectively. This assumption is not correct, and the nature of the errors that it can cause are best illustrated by considering a " soaking-out " experiment, i.e., one in which the tissue is first left in a solution containing the labeled ion until equilibrium is reached (specific activity the same in all compartments) and is then transferred (at $t=0$) to a large volume of unlabeled solution. The total radioactivity of the tissue (P_2+P_3) is followed and is found to consist approximately of two exponential terms.

$$P_2+P_3=Ae^{-\lambda_1 t}+Be^{-\lambda_2 t}$$

The common assumption is equivalent to writing $P_2=Ae^{-\lambda_1 t}$ and $P_3=Be^{-\lambda_2 t}$, while in fact both the intracellular and the extracellular components contain terms with each of the rate constants.

$$P_2=a_2e^{-\lambda_1 t}+b_2e^{-\lambda_2 t} \tag{B 1}$$

and

$$P_3=a_3e^{-\lambda_1 t}+b_3e^{-\lambda_2 t} \tag{B 2}$$

What we require is (a_3+b_3), the initial value (P_{30}) of the intracellular radioactivity P_3; this may be obtained from the observed quantities A, B, λ_1, and λ_2 as follows (a steady state as regards fluxes and total concentrations is assumed throughout):

1. At $t=0$, the specific activity is equal in the intracellular and extracellular compartments and therefore $dP_3/dt=0$, i.e., by differentiating Eq. (B 2)

$$\lambda_1 a_3+\lambda_2 b_3=0 \tag{B 3}$$

whence

$$P_{30}=(a_3+b_3)=b_3[(1-(\lambda_2/\lambda_1)] \tag{B 4}$$

2. The over-all rate of loss of radioactivity is always equal to $k_{21}P_2$ (the bathing solution is assumed to be so large that return of radioactivity from it may be neglected). At $t=0$, $P_{20}=a_2+b_2$, and the rate of loss of radioactivity is $A\lambda_1+B\lambda_2$. Therefore,

$$k_{21}(a_2+b_2)=A\lambda_1+B\lambda_2 \tag{B 5}$$

At long times, where $e^{-\lambda_1 t}$ can be neglected in comparison with $e^{-\lambda_2 t}$, $P_2=b_2e^{-\lambda_2 t}$ and the rate of loss of radioactivity is $\lambda_2 Be^{-\lambda_2 t}$. Therefore

$$k_{21}b_2e^{-\lambda_2 t}=\lambda_2 Be^{-\lambda_2 t}$$

or

$$k_{21}=\lambda_2 B/b_2 \tag{B 6}$$

Substituting Eq. (B 6) into Eq. (B 5)

$$(a_2+b_2)\lambda_2 B/b_2=A\lambda_1+B\lambda_2 \tag{B 7}$$

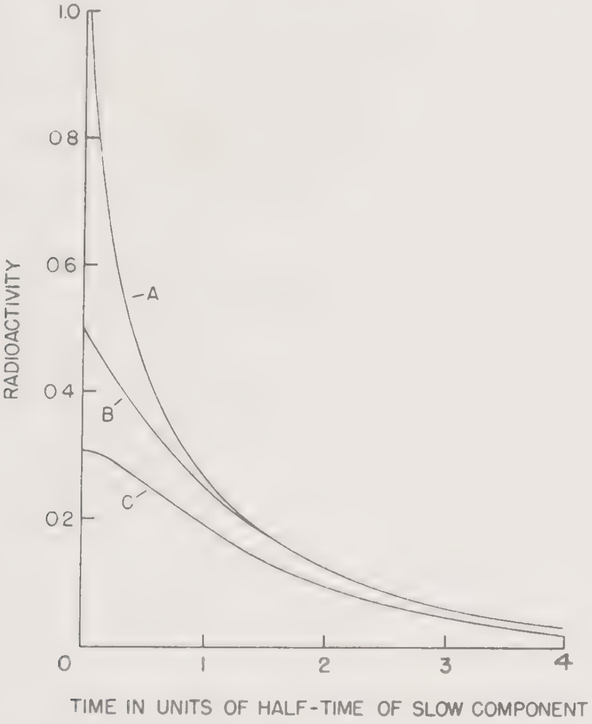
3. Combining Eqs. (B 3) and (B 7), and remembering that $A = a_2 + a_3$ and $B = b_2 + b_3$, we obtain

$$b_3 = \frac{AB\lambda_1(\lambda_1 - \lambda_2)}{A\lambda_1^2 + B\lambda_2^2}$$

whence, using Eq. (B 4)

$$P_{30} = \frac{AB(\lambda_1 - \lambda_2)^2}{A\lambda_1^2 + B\lambda_2^2} \tag{B 8}$$

The simple assumption $P_{30} = B$ is therefore an overestimate; the error can be very serious as may be seen by considering the case where the fast and slow components are of equal magnitude ($A = B$) and one is three times as fast as the other ($\lambda_1 = 3\lambda_2$). Equation (B 8) gives $P_{30} = 0.4B$, so that the simple assumption $P_{30} = B$ overestimates the intracellular material by a factor of 2.5.



A = Total radioactivity remaining ($P_2 + P_3$)
B = Slow component ($Be^{-\lambda_2 t}$)
C = Intracellular component ($a_3e^{-\lambda_1 t} + b_3e^{-\lambda_2 t}$)

FIG. 25. Diagram showing the time-course of efflux of radioactivity from tissue previously soaked in radioactive material, as discussed in Appendix 2.

Figure 25 shows the time-courses of total radioactivity and of intracellular radioactivity for the case $A = B$, $\lambda_1 = 5\lambda_2$. The slow component $(b_2 + b_3)e^{-\lambda_2 t}$ is also shown.

Analogous formulas involving only the observed time-course of loss of radioactivity can also be derived for cases where the resistance to escape from the extracellular spaces is due to diffusion and is distributed through the thickness of the tissue. The specific activity of the extracellular fluid is not uniform in these cases, but varies with distance from the surface in the way described by Keynes (1954).

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CHAPTER 6

Ion Transport

VALBORG KOEFOED-JOHNSEN AND HANS H. USSING

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I. Definition

An important aspect of the problem of growth and maintenance of life is the permeability of cells to substances in the medium surrounding them. One of the most puzzling features of this problem is the ability of nearly every living organism to maintain a constant cellular composition of a series of inorganic ions at concentrations differing greatly from those in the surrounding medium. A well-known example is the ability of most animal and plant cells to accumulate potassium and exclude sodium. Not only are cells able to maintain a constant ionic level, but cellular elements like mitochondria have also been shown to possess the ability to regulate their ionic composition, even though the limiting membranes are permeable to the ions in question.

During the last ten to fifteen years the general availability of radioactive isotopes of almost every inorganic ion has added considerably to our knowledge about ion transfer across cell boundaries. Our knowledge, though far from complete, allows a formal classification of transfer processes.

Transport of a substance across a biological membrane is called passive if the process can be accounted for by means of ordinary physical forces: those caused by a concentration gradient, an electrical gradient, a solvent drag, or any combination of these. Active transport processes, then, are those which cannot be explained as the result of these forces, but involve participation of some energy-yielding chemical reaction. Active transport processes have commonly been classified (Ussing, 1948, 1949b, c; Rosenberg, 1948, 1954) as those where the substance in question is moved against an electrochemical gradient; in other words, the substance gains free energy by the transferring process. This extra free energy is delivered by the metabolism of the cell. However, an active mechanism which under certain conditions moves an ion species against the electrochemical gradient should, under other conditions, be able to assist the diffusion of the same ion species in the direction of the electrochemical gradient. It would be consistent for this downhill process to be called active transport as well, but it is questionable whether it is possible to demonstrate experimentally an active component in downhill processes. The inner nature of active transport processes has still not been fully explained, but it is generally believed that chemical reactions with membrane constituents are necessarily involved. Whether the same or different mechanisms are responsible for the various cases of active transport is still an open question that is left for solution to future biochemical investigation.

II. Experimental Characterization

A. Uphill Transport

The demonstration of uphill transport (i.e., in the case of an uncharged substance, transport from a lower to a higher chemical potential, and in the case of a charged substance, transport from a lower to a higher electrochemical potential) has generally been used as a criterion of active transport, as this definition is based solely on experimental evidence and does not involve assumptions concerning the membrane in question.

The demonstration of such an uphill transport of an ion species through a cell membrane requires knowledge about the extra- and intracellular concentrations of the ion in question and about the electrical potential across the cell membrane, as measured by means of a microelectrode. Such information makes possible calculation of the electrochemical potential, if one assumes that the activity coefficients in the two phases are the same and that the potential determined by the microelectrode is thermodynamically significant.

A given ion will then be in equilibrium with respect to a given membrane when its electrochemical potential in the outside solution, $\bar{\mu}_{(o)}$, equals its electrochemical potential in the inside solution, $\bar{\mu}_{(i)}$. The electrochemical potential of the jth ion is defined by the equation

$$\bar{\mu}_j = RT \ln a_j + zF\psi + I \quad (1)$$

where R, T, z, F have the conventional meaning, a denotes chemical activity, ψ, electrical potential, and I, a constant. Deviations from the equilibrium condition

$$\bar{\mu}_{j(o)} = \bar{\mu}_{j(i)} \quad (2)$$

are indications that the ion is subject to active transport.

The equilibrium analysis has proved very useful in demonstrating, for example, the important fact that potassium in skeletal muscle is close to electrochemical equilibrium. However, the method has certain limitations, the main one being that most biological systems are not in an equilibrium state, but change their ionic composition continuously. Furthermore, it may turn out to be quite erroneous to assume that the activity coefficients are identical in the two phases, as the ion may be partly bound in a complex in the cell interior. Consequently, it is only by a process of elimination that a given ion movement can be described as active transport. That is to say, it must be shown unambiguously that the behavior of the substance cannot be explained as a result of ordinary physical forces. It is therefore necessary to know how a substance behaves if treated passively.

B. Flux Ratio Analysis of the Physical Forces

1. *Diffusion*

It has become common usage to call passive transfer of a substance " diffusion ", if brought about by differences in either chemical or electrical potential or caused by solvent drag (i.e., the force which arises from the flow of the solvent through a porous membrane and which acts on a diffusing molecule in such a way that diffusion of the molecules is enhanced in the direction of the flow, while diffusion against the stream is impeded). Basically, however, diffusion is the movement caused by the thermal energy of the particles.

The quantitative laws of diffusion for uncharged particles in a steady-state system were formulated by Fick (1855). Fick's well-known first law is (cf. Vol. I, Chapter 2):

$$M = -(A/g)c(d\mu/dx) \quad (3)$$

where M is the flux or amount of substance crossing a unit area perpendicular to the direction of diffusion in unit time, g is the frictional coefficient, A is the area available for diffusion in the x direction, $d\mu/dx$ is the chemical potential gradient, and c is the concentration of diffusing substance.

2. *Electric Force*

In analogy with Fick's law, the following expression is valid for a charged particle (disregarding solvent drag as was done in the case of Fick's law, above).

$$M_j = -(A/g)c_j(d\bar{\mu}_j/dx) \tag{4}$$

The force acting on the jth ion, $-d\bar{\mu}_j/dx$ (the electrochemical potential gradient), can be resolved into two components: the force of diffusion, $-d\mu_j/dx = -RT(d \ln a_j/dx)$, and the electric force, $-z_jF(d\psi/dx)$. A third force due to a possible difference in hydrostatic pressure may be added, $-V_j(dp/dx)$, where V_j is the molar volume and p the pressure. Equation (4) can then be rewritten as follows:

$$M_j = -\frac{ARTc_j}{g}\left[\frac{d \ln c_j}{dx} + \frac{d \ln f_j}{dx} + \frac{V_j}{RT}\left(\frac{dp}{dx}\right) + \frac{z_jF}{RT}\left(\frac{d\psi}{dx}\right)\right] \tag{5}$$

where f is the activity coefficient. However, there is no difference between the hydrostatic pressures on each side of the membrane in most of the biological systems treated here and consequently the pressure term will be left out in what follows.

In order to integrate this differential equation for an ion moving under the influence of chemical and electrical gradients, it is necessary to make certain assumptions concerning the membrane. Most solutions given in the literature have been obtained by first assuming that the membrane is uniform throughout, i.e., that A, the area available for diffusion, and g, the coefficient of friction, do not vary with x. The solution given by Goldman (1943) is further based upon the assumption that the electrical potential changes linearly through the membrane, in other words, that the electric field is constant. Teorell (1951), in his treatment, chose to consider the concentration gradient through the membrane to be linear.

However, Ussing (1949b), and at the same time Teorell (1949), pointed out that a solution can be obtained without these assumptions if the ratio between influx and efflux is considered, rather than the flux itself. As A, g, f, and ψ vary with x in the same way for the same ions, these variables cancel out in the expression for the flux ratio, which now can be written, when integrated between the boundaries $x = x_0$ and $x = 0$, as

$$M_{j(\mathrm{in})}/M_{j(\mathrm{out})} = \bar{a}_{j(\mathrm{o})}/\bar{a}_{j(\mathrm{i})} \qquad (6)$$

or

$$RT \ln (M_{j(\mathrm{in})}/M_{j(\mathrm{out})}) = \bar{\mu}_{j(\mathrm{o})} - \bar{\mu}_{j(\mathrm{i})} \qquad (7)$$

where $M_{j(\mathrm{in})}$ is the influx, $M_{j(\mathrm{out})}$ the efflux, $\bar{a}_{j(\mathrm{i})}$ the electrochemical activity of the ion in the inside solution, and $\bar{a}_{j(\mathrm{o})}$ its electrochemical activity in the outside solution. This equation is valid for an ion moving passively under the influence of diffusion and electric forces, but in the absence of solvent drag. The individual fluxes can be determined by means of tracers, either simultaneously, if the ion in question has two suitable isotopes, or else separately. The electrochemical activity ratio can be calculated from the inside and outside concentrations of the ion and from the measured electrical potential. Agreement between these two estimates of the flux ratio means that the ion in question moves passively through the membrane examined.

By means of this analysis it has been shown, for example, that potassium behaves passively in the squid axon (Keynes, 1951) and in crab nerve (Keynes and Lewis, 1951), and that chloride moves passively through the isolated frog skin (Koefoed-Johnsen *et al.*, 1952a). (However, the agreement in squid axon has proved to hold only at high external K concentrations, whereas an active absorption of K ions prevailed at low outside K concentrations [cf. Hodgkin and Keynes, 1955b].)

Flux ratios smaller than the activity ratios indicate that the substance is subject to what has been called exchange diffusion (Ussing, 1947). By this is meant a one-to-one exchange of the same ion species across a cell border, without any net change. This phenomenon can be detected only by means of isotopes. Exchange diffusion is supposed, like active transport, to be carrier-mediated but different from it in that no work is performed ; consequently the process needs no special energy supply from metabolism.

Exchange diffusion of sodium has been reported in muscle (Ussing, 1947 ; Swan and Keynes, 1956) and in kidney mitochondria under certain conditions (Davies, 1954). In duck red blood cells, there is an exchange diffusion of potassium which is even larger when the aerobic metabolism is abolished by poisons like fluoride (Tosteson, 1955). Hogben (1951) demonstrated an exchange diffusion component in the chloride transport across the mucosa of the frog stomach. There is a large exchange diffusion of phosphate in *Micrococcus pyogenes* (Mitchell, 1953). An exchange diffusion where the carrier involved cannot distinguish between Na and K was found by Croghan (1958) in the brine shrimp *Artemia salina*.

A flux ratio bigger than the activity ratio is found in the special case of passive transport described by Hodgkin and Keynes (1955b) and called by them " single file diffusion ". They found that potassium in a squid axon poisoned with dinitrophenol behaved according to the equation

$$M_{\text{in}}/M_{\text{out}} = (\bar{a}_{(\text{o})}/\bar{a}_{(\text{i})})^n$$

where $n = 2$ or 3, as if potassium, when crossing the membrane, had 2 or 3 positive charges. They explained this behavior by assuming that 2 or 3 K ions move as a unit, because the only paths in the membrane through which potassium can move have 2–3 sites, each of which has to be occupied by a K ion.

In the cases just discussed, where the behavior of the ions differs from that predicted by Eq. (6), the net transfer, if any, must be downhill, if the transport in question is to be considered passive.

To sum up, deviations from Eq. (6) cannot always be taken as indications of active transport. However, where the electrochemical activities of the inside and outside solutions are identical, a net transfer of the ion can only be accounted for by active transport.

3. *Solvent Drag*

Thus far, in the treatment of passive behavior of ions, we have disregarded the effect of solvent drag, i.e., the force which the movement of the solvent exerts on the diffusing particles so that molecules moving in the direction of flow are speeded up, whereas those moving against the flow are slowed down. If we are dealing with a porous membrane through which water forms a continuous phase, an additional term has to be added to the diffusion equation [Eq. (5)] to take account of solvent drag.

This force can be expressed as

$$-[(dP/dx)(G_j/g'_w)]$$

i.e., the gradient of the hydrostatic or osmotic pressure through the membrane times the ratio between the friction coefficients G_j (the friction between the jth ion and water) and g'_w (the friction between the pore wall and water). The expression may be put equal to

$$-RT(d \ln a_w/dx)(G_j/g'_w)$$

where a_w is the activity of water.

The general differential equation for an ion moving under the influence of diffusion, electric force, and solvent drag may then be written as follows :

$$M_j = -\frac{ARTc_j}{g}\left[\frac{d \ln c_j}{dx} + \frac{d \ln f_j}{dx} + \frac{z_j F}{RT}\left(\frac{d\psi}{dx}\right) + \frac{d \ln a_w}{dx}\left(\frac{G_j}{g'_w}\right)\right] \qquad (8)$$

G_j, the frictional coefficient between solute and solvent, is related to the free diffusion coefficient D_j of the jth ion by the expression

$$G_j = RT/D_j \tag{9}$$

While the linear rate of flow $-(dP/dx)(1/g'_w)$ may vary with x, the same is not true for the volume rate of flow Δ_w, which is given by

$$\Delta_w = M_{w(\mathrm{in})} - M_{w(\mathrm{out})} = A\,(1/g'_w)\,(dP/dx) \tag{10}$$

where A is the area available for flow.

As previously in the expression for the flux ratio, most of the unknowns in Eq. (8) cancel out. Substituting Eqs. (9) and (10) and integrating gives

$$\ln \frac{M_{j(\mathrm{in})}}{M_{j(\mathrm{out})}} = \ln \frac{\bar{a}_{j(\mathrm{o})}}{\bar{a}_{j(\mathrm{i})}} + \frac{\Delta_w}{D_j} \int_0^{x_0} \frac{1}{A}\, dx \tag{11}$$

This integral cannot be evaluated directly. It is, however, independent of the substance in question and has the same value for a charged as for an uncharged molecule, so that, provided the molecule is not actively transported, the integral can be evaluated by means of a suitable test substance. This substance should be absolutely hydrophilic (it might even be water itself), since the validity of the method is dependent upon the fact that solute and solvent follow the same paths through the membrane.

The effect of solvent drag is no doubt important for those biological systems where osmotic and hydrostatic gradients occur. But when a transfer process is examined for active or passive behavior, solvent drag may generally be safely ignored, at least as a first approximation. On the other hand, the demonstration of a sizable drag effect on a test substance can be used to indicate the presence of pores in a membrane. [For further discussion of solvent drag see Pappenheimer *et al.* (1951), Koefoed-Johnsen and Ussing (1953), Ussing and Andersen (1956), discussion of the Faraday Society on Membrane Phenomena (1956), Andersen and Ussing (1957), Garby (1957), and Meares and Ussing (1958)].

III. Types of Active Transport

A. Anion Transport

The interest in ion transport has been mainly focused on the behavior of cations, which in animal cells, at least, seem to be the ions actively transported, while the anions follow passively. Conceivably, both anions and cations might be transported actively at the same time, but in principle an active salt accumulation requires the movement of only one ion species. In the latter case, the ion of opposite sign will then follow the electrical gradient set up by the partner. Thus, in the isolated frog skin, chloride ions move passively (Koefoed-Johnsen *et al.*, 1952a). However, treatment with adrenaline induces an outward-directed transport of chloride, which probably originates in the skin glands (Koefoed-Johnsen *et al.*, 1952b). The living frog seems to have an active chloride transport, and is able to take up chloride from tap water (Krogh, 1937 ; Jørgensen *et al.*, 1954). While the mechanism for active cation transport is generally rather specific, that for anion transport seems to be less so. For example, Krogh (1937) demonstrated that Br^- was taken up when substituted for chloride, that NO_3^- and SCN^- penetrate easily, though not necessarily actively, and that I^- and SO_4^{2-} are virtually nonpenetrating. The ability to take up chloride from dilute solutions is not unique for the frog, but occurs in many fresh water animals (Krogh, 1939). It is not yet known whether these cases involve an active transport of chloride, as the electrochemical potential gradient of this ion has not been measured.

On the other hand, a clear-cut case of active chloride transport was demonstrated by Hogben (1951) in the mucosa of the frog stomach. By means of the short-circuiting technique (Fig. 1 ; Ussing and Zerahn, 1951), Hogben (1951) showed that an active transfer of chloride from the nutrient to the secretory side could account for the short-circuit current drawn from the isolated nonsecreting mucosa. The mechanism will transport bromide as well, and at an even higher rate (Heinz *et al.*, 1954). In the same context it is interesting to note that the thyroid gland, which accumulates iodide (Vanderlaan and Vanderlaan, 1947), will also concentrate chloride and bromide (Bauman and Metzger, 1949), although not to the same degree as iodide.

The sublingual salivary gland shows behavior which, judged from intra- and transcellular potential measurements, is compatible with a chloride transport (Lundberg, 1957a, b ; see also Vol. I, Chapter 10). The transporting mechanism does not distinguish between chloride and bromide ions, and nitrate seems also to be actively transported, but to a much smaller degree.

The kidney does not reabsorb anions actively *in vivo*, but Deyrup and Ussing (1955) have given evidence of an active accumulation of sulfate ions in kidney slices.

Active uptake of anions, such as chloride and nitrate, seems to occur generally in plant cells, for example in the giant cells of characeans and algae. *Halicystis ovalis* shows a potential difference of about 70 mv across its cell membrane (inside negative) when both the inside and outside solutions are sea water (Blinks, 1940). That this negative potential could be attributed to an active extrusion of sodium has in fact been demonstrated by Blount (1957) by means of the short-circuiting technique, but his results gave evidence for an additional active transport of chloride inwards as well. MacRobbie and Dainty (1958) arrived at the same result for *Nitellopsis* from considerations of the electrochemical potentials of the various ions.

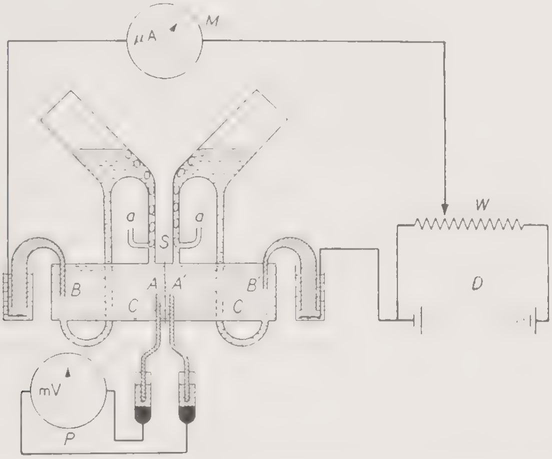

FIG. 1. Diagram showing the apparatus used for short-circuiting the skin. The skin *S* is mounted between two celluloid or lucite chambers *C* containing Ringer solution. The potential difference across the skin is read on the potentiometer *P* which is connected through calomel electrodes to two agar-Ringer bridges *A* and *A′*, which open close to the skin. Another pair of agar-Ringer bridges *B* and *B′*, opening at a distance sufficient to give a homogeneous electric field at the level of the skin, are connected through silver/silver-chloride electrodes with a microammeter *M* and a battery *D*. The current in this circuit is adjusted by means of a variable resistance *W* so that the potential drop across the skin is zero ; this accomplishes a total short circuit of the skin.

Higher plants also take up cations and anions from dilute solutions, although not necessarily in equivalent quantities. Excised roots of cereals are favorite experimental plant material (Hoagland and Davis, 1923 ; Hoagland and Broyer, 1936 ; Lundegårdh and Burström, 1933), and in them evidence was found of transfer across several cell membranes, from the outside medium into the central vessel (bleeding sap). Other types of material used extensively are tissue slices of various storage organs, for example, carrots, potatoes, and beets (Steward, 1937, Robertson, 1941), which illustrate a transfer of substances across the cell membrane and protoplasm into the cell sap in the vacuole. Lundegårdh (1938) assumes that an anion transport is taking place, and his potential measurements showed in fact that the bleeding sap is negative with respect to the outside. The concentration of anions against this potential difference is consequently active. Furthermore, the potential difference is big enough to account for most of the potassium absorption. Hoagland and Steward (1939, 1940), on the other hand, favor the view that both cation and anion transport occur in higher plants. As direct measurements of the electrochemical activities of the ions have not been made in general, it is impossible to settle the question whether anions, cations, or both are actively transported, all three viewpoints having their supporters. There is disagreement not only on how many steps (active and inactive) are involved in the concentration, but also with which morphological structures the selective barrier (or barriers) of the different stages should be associated. Out of the discussion has grown the concept of " apparent free space " (Briggs and Robertson, 1957), a term that covers the volume of tissue which is accessible for " free diffusion and ion exchange ", i.e., a Donnan phase.

B. Cation Transport

The fact that potassium ions predominate over sodium ions intracellularly, while the reverse is true extracellularly, has led to extensive studies of the behavior of these cations.

The introduction of isotopes into biology involved a reconsideration of older theories about ion accumulation, based as they were on assumptions of impermeability. However, the use of isotopes has revealed that true impermeability is rare and has made necessary reinterpretation of older findings.

The distribution of potassium and sodium could be accounted for by the active transport of only one of them, the other then being in electrochemical equilibrium across the membrane. This explanation seems to hold for many tissues and cells. An increasing amount of experimental evidence has shown that ordinarily the sodium ion is actively transported, while the potassium ion is more or less in electrochemical equilibrium. Muscles thus probably keep their sodium concentration down by means of the outwardly directed " sodium pump " (Dean, 1941) and thereby create the necessary negative potential inside to insure the high internal potassium concentration (Steinbach, 1940; Krogh, 1946; Hodgkin, 1951). Sodium extrusion has also been demonstrated, for example, in giant nerve axons (Hodgkin, 1951), blood cells (Danowski, 1941; Harris, 1941), brain slices (Davies, 1954), kidney and liver slices (Mudge, 1951, 1953, 1954; Davies, 1954), and mitochondria (Davies, 1954; Mudge, 1954, 1955). Net transport of sodium across the surviving isolated frog skin from the outside to the inside bathing solutions was demonstrated by Ussing (1948). By combined measurements of the short-circuit current and of influx and efflux of sodium, Ussing and Zerahn (1951) concluded that the net flux of sodium could account for all the current drawn from the skin, i.e., that sodium is the only ion subject to active transport. Determinations of the net sodium flux by means of double labeling (i.e., the simultaneous measurement of influx and efflux of sodium by means of the two isotopes Na^{24} and Na^{22}) along with measurements of the current drawn from the skin confirmed this view unequivocally (Koefoed-Johnsen *et al.*, 1952b).

Recent research on ion movements in many tissues and cell types has yielded evidence in several instances that sodium extrusion is not an independent process, but seems to be more or less firmly linked to potassium accumulation. Thus Harris and Maizels (1951) found that the sodium efflux in human red cells is reduced when the external potassium concentration is low or if there is no potassium present at all, and proposed a linkage between Na efflux and K influx. The subject of the movement of alkali metal ions across the red cell membrane has been recently reviewed by Tosteson (1955), who concludes that K efflux and Na influx are passive and that the active Na efflux and K influx are probably linked. This is in contrast to Solomon (1952), who suggested different carrier processes for each of the four ion movements. The problem has been reinvestigated by Glynn (1956, 1957) who concludes that the active fluxes, Na efflux and K influx, are closely linked.

A linkage between Na extrusion and K influx has also been demonstrated in frog muscles by Keynes (1954) and in *Sepia* nerve axons by Hodgkin and Keynes (1955a). The transport of sodium across the isolated frog skin is also dependent on the presence of potassium in the bathing solutions (Koefoed-Johnsen, 1955) and is very much reduced in the absence of potassium. Again there seems to be a linkage between sodium and potassium transport. (This will be discussed in more detail below.)

Active transport of sodium can occur not only in exchange for potassium, but also in exchange for hydrogen ions. This seems to be the case in kidney tubules, where the necessary quantity of H^+ is generated from carbonic acid by carbonic anhydrase, from CO_2 released in the cells during their respiration (Pitts, 1948; Berliner *et al.*, 1950). If hydrogen ions are not available (as after the administration of carbonic anhydrase inhibitors), K^+ will replace H^+ in the exchange reaction (Berliner *et al.*, 1951; see also Vol. I, Chapter 9).

Although there is increasing evidence for the view that a linkage exists between the active movements of sodium and potassium, there are instances where active sodium extrusion and potassium accumulation occur independently. In *Ulva lactuca*, for example, these processes seem to have separate mechanisms, as they can be inhibited separately

(Scott and Hayward, 1954). Likewise Koch and Evans (1956) report the existence of two separate mechanisms for sodium and potassium absorption through the gills of the crab, *Eriocheir sinensis*. However, their evidence is not conclusive, but could equally well be interpreted in terms of a common mechanism which does not discriminate between Na^+ and K^+.

Ehrlich mouse ascites cells are able to accumulate potassium and extrude sodium against the electrochemical gradients of both ions (Hempling, 1958; Maizels *et al.*, 1958a, b, c). Both groups of authors agree that two separate transport mechanisms are operative, although the transport of the two ions seems to be linked under steady-state conditions in the absence of glucose and at room temperature, where the net fluxes of both ions are equal. Cooling to 2° C (Hempling, 1958) caused a depletion of potassium while sodium enters the cell. The loss of potassium was, however, much faster than the entry of sodium; raising the temperature to 22° C and addition of glucose enhanced the K accumulation sevenfold, while sodium was uninfluenced by glucose. Hempling does not believe that the potassium-hydrogen exchange, so characteristic of yeast cells (Conway and O'Malley, 1946; Rothstein and Enns, 1946), can account for the fraction of K accumulation not taken care of by sodium, on the grounds that net flux experiments in the absence of glucose and glycolysis did not change the picture.

Yeast cells represent a thoroughly studied example of cation transport in plants. During the fermentation of an unbuffered glucose solution in the presence of KCl, but in the absence of phosphate, yeast cells take up potassium ions in a stoichiometric exchange for hydrogen ions derived primarily from metabolic reactions (Conway and O'Malley, 1946; Rothstein and Enns, 1946). Part of the accumulated potassium is balanced against metabolic anions such as succinate and bicarbonate, but part is at the expense of cellular buffering capacity, with a resulting alkalization of the cytoplasm. The pH of the suspending medium is correspondingly reduced to low values. If no KCl is present in the medium, acid, as succinic acid, is still excreted, although in reduced quantity. If phosphate is present, but not potassium, phosphate is absorbed to a limited extent only. Sodium can compete in the hydrogen-potassium exchange process, but has to be present in a concentration 25 times that of potassium for equal uptake. Rubidium is taken up at about half the rate of potassium, while both cesium and lithium behave like sodium. If the fermenting yeast cells are in a medium with no KCl, but with sodium citrate instead, they will take up Na^+, presumably by means of the K carrier, as even a small amount of K^+ in the outside medium will prevent the Na^+ uptake (Conway and Moore, 1954). When sodium has been accumulated, it is actively extruded by a special

Na carrier which, according to Conway (1954), is more specific towards Na^+ than is the K carrier towards K^+.

From the foregoing there appears to be an increasing amount of evidence indicating that the " sodium pump," so widely distributed in living cells, is in many instances an active sodium-potassium exchange mechanism (where H^+ might be able to substitute for K^+), and that such an active exchange could account for the high intracellular K/Na ratio found in most cells.

IV. Occurrence of Active Transport

The increasing interest in active transport processes has resulted in an intensive investigation of transfer phenomena in a large variety of cells and tissues. Table I thus gives a list of some well established cases of active transport into and out of different cell types, while Table II shows some good examples of transport across cells. The tables are not

TABLE I

TRANSPORT INTO AND OUT OF CELLS†

Material	Na	K	H	Cl	I	Phosphate
Red cells						
Human	1, 2	3				
Chicken	4	4				
Leucocytes	5	6				
Ehrlich ascites cells	7, 8	7, 8				
Giant nerve axon	9	9				
Muscle fiber	10	11				
Yeast	12	13	14, 15			
Algae						
Ulva lactuca	16	16				
Halicystis ovalis	17			17		
Nitellopsis	18			18		
Staphylococcus aureus						19

† Numbers indicate references set below table.

1. Danowski (1941)
2. Harris (1941)
3. Harris and Maizels (1951)
4. Maizels (1954)
5. Wilson and Manery (1949)
6. Pulver and Verzar (1941)
7. Maizels *et al.* (1958b)
8. Hempling (1958)
9. Hodgkin and Keynes (1955a)
10. Steinbach (1940)
11. Keynes (1954)
12. Conway *et al.* (1954)
13. Pulver and Verzar (1940)
14. Conway and O'Malley (1946)
15. Rothstein and Enns (1946)
16. Scott and Hayward (1954)
17. Blount (1957)
18. MacRobbie (1957)
19. Mitchell (1954)

TABLE II

Transport across Cells†

Material	Na	K	H	Cl	I	Phosphate
Stomach (frog)				1		
Rumen (sheep)	2					
Ileum (rat)	3			3		
Colon (toad)	4					
Caecum (guinea pig)	4					
Kidney	5	6	7			8
Urinary bladder (toad)	9					
Seminal vesicle mucosa	10	10				
Thyroid gland					11	
Salivary gland				12		
Skin (frog)	13					
Skin after adrenaline				14		
Gill (eel)				15		
Gill (*Eriocheir sinensis*)	16	16				
Malpighian tubule of insects		17				
Plants						
Roots of cereals				18		
Disks of storage organs				19		

† Numbers indicate references set below table.

1. Hogben (1951)
2. Dobson (1955)
3. Curran and Solomon (1957)
4. Ussing and Andersen (1956)
5. Mudge *et al.* (1949)
6. Berliner *et al.* (1951)
7. Pitts (1948)
8. Pitts and Alexander (1944)
9. Leaf (1955); Leaf, *et al.* (1958)
10. Breuer and Whittam (1957)
11. Vanderlaan and Vanderlaan (1947)
12. Lundberg (1957a, b)
13. Ussing (1948)
14. Koefoed-Johnsen *et al.* (1952b)
15. Keys (1931)
16. Krogh (1938)
17. Ramsay (1953)
18. Lundegårdh (1945)
19. Robertson and Wilkins (1948)

intended to include all known cases of active ion transport. However, some illustrative and characteristic examples showing different approaches in demonstrating active ion transport have been emphasized.

V. Hypotheses of Transport Mechanisms

That active ion transport processes are involved in the numerous cases where ion accumulation and extrusion occur against their electrochemical potentials is beyond doubt; and that the required energy is derived from metabolism, aerobic or anaerobic, has also been unequivocally proved. But how and in what form the energy is transferred to the transported ion is still unknown. There are several theories and model systems which could account for ion transport, but so far none has been proved to work in any particular case.

A few examples of the commonly invoked theories will be given below.

A. Carrier Hypothesis

The carrier hypothesis dates back to Osterhout's classical guaiacol models (1933, 1940). In its simplest form it involves the assumption that a substance in the membrane forms a specific complex with the ion at one border of the membrane, which then can diffuse through the membrane. A gradient is maintained by constant destruction of the complex at the other border of the membrane, so that the movement of the ion complex, as distinct from the ion, is downhill. The energy fed into the system would then be used to move the complex across the membrane or to form and destroy the carrier at the respective borders.

As cell membranes are generally supposed to be composed largely of phospholipids, it has been an implicit assumption in many carrier hypotheses that the ion complex is lipid soluble. This idea involves the binding of the ion to some chelating substance. Alkali metals do not readily form chelate complexes which are fat soluble. But some have been reported in the literature, for example by Schwarzenbach and Ackermann (1947), by Rosenberg (1954), and by Christensen (1955). Although chelating agents usually show a slight preference for one or another cation, this does not suffice to explain the strong discrimination between Na and K exhibited by most cell membranes.

If ion transport occurs through a minor fraction of the cell surface, this fraction need not necessarily be composed of lipids, but could just as well be protein. The demonstration of the existence of pores in several membranes, where an osmotic flow takes place, strongly suggests that these pores are not of lipid nature. The possibility remains that ion transport somehow occurs through these water-filled channels, as for example through the pores of an ion exchange resin. The selectivity of cation exchange resins has been discussed by Bregman (1953), among others, but the degree of discrimination between Na and K achieved is too low to account for the events in biological membranes.

For a detailed analysis of a carrier model system, see Patlak (1956 and 1957).

B. Fluid Circuit Hypothesis

This theory was put forward by Ingraham *et al.* (1938) as an attempt to explain salt absorption in the gut (see Vol. I, Chapter 8). The implicit assumption of the theory is the existence in the membrane of structures with different permeabilities towards water, monovalent salts, and divalent salts. It is assumed that through one set of larger pores (permeable to water and monovalent salts) a salt solution is carried under the influence of electroosmosis, while pure solvent is returning through another set of pores, which are so small that they are almost impermeable towards ions, but permeable to water.

In principle a kind of solvent drag could produce transport of salt, but it remains to be explained how specificity can be obtained.

C. Electron-Linked Transport Hypothesis

These theories are based on the idea that the energy of the electron-transferring reactions of respiration can be used directly for ion transport, as it is known that active ion transport is in most cases dependent on aerobic metabolism. Three very similar theories have been proposed by Davies and Ogston (1950) and Davies and Krebs (1952), by Conway (1953a; the " redox pump "), and by Lundegårdh (1945; the theory of " anion respiration " in plants).

The theoretical basis for the three hypotheses is the assumption that the iron in the cytochromes undergoes cyclic oxidation and reduction during respiration, while electrons are passed along a chain of the cytochromes. The basic reaction used for the coupling of ion transport to the respiratory system is the change in valency of the Fe atom.

$$4\ Fe^{3+} + 4(H) \rightarrow 4\ Fe^{2+} + 4\ H^+$$
$$4\ Fe^{2+} + O_2 + 2\ H_2O \rightarrow 4\ Fe^{3+} + 4\ OH^-$$

If the terminal cytochromes of the chain are spatially separated, it should be possible to obtain a separation of H^+ and OH^- too. By exchanging these for other ions, active transport of the exchanging ions might occur. The anions would then be transported in a direction opposite to that of the electrons, that is, from the medium with the higher oxidizing power to the medium with the lower oxidizing power.

One of the difficulties of this theory, which it shares with other theories, is the failure to explain the specificity of ion transport. In the " redox pump " theory, Conway (1953a) has overcome this difficulty by assuming that the cation forms a complex with the reduced form of a respiratory enzyme, crosses the cell membrane in this form, and is released by oxidation at the other side of the membrane.

Energy considerations constitute a more serious objection to a process involving direct utilization of the energy of respiratory electron transfer for active transport. If active ion transport should proceed according to an electron-transferring theory, then a maximum of 4 equivalents of ion could be transported per molecule of oxygen consumed according to the reaction

$$4\ H^+ + 4e + O_2 \rightarrow 2\ H_2O$$

There is, however, clear evidence that the ratio of the number of equivalents of ions to the number of molecules of O_2 can exceed 4. Such values have been obtained in frog gastric mucosa (Crane and Davies, 1951), in the isolated frog skin (Zerahn, 1956; Leaf and Renshaw, 1957), and in plants (Handley and Overstreet, 1955; Sutcliffe, 1957, Sutcliffe and Hackett, 1957).

These findings strongly indicate that the energy derived from respiratory systems through the intermediary of an electron-transferring process is not used directly for ion transport. It seems quite likely, as discussed below, that the energy for ion transport is provided by energy-rich phosphate bonds.

D. Other Hypotheses

Various general mechanisms based on enzyme reactions have been proposed, for example, that phosphorylation participates somehow in the transport process. Goldacre (1952) has suggested that a contractile protein in the membrane might act as a carrier. He proposed that by coiling and uncoiling of peptide chains, the protein could alternately adsorb and desorb ions in the following way. When the protein unfolds, the side chains (hydrogen bonds, salt linkages) which keep the polypeptide chains in their folded configuration will be pulled apart and a number of sites equal to the number of broken bonds will become available for the adsorption of ions. When the protein refolds, the side chains will again be attached and the ions desorbed. The folding-unfolding cycle is supposed to be driven by adenosine triphosphate, in analogy to the actomyosin-ATP system.

Danielli (1954) and Stein and Danielli (1956) discuss various modifications of Goldacre's contractile protein hypothesis. They assume that the cell membrane consists of a lipoidal layer with protein layers on either side, which may or may not form a regular lattice. The idea of contraction and relaxation of a protein is used for a " propelled carrier " mechanism, where the ions to be transported are attached to an adsorption center of the membrane, which in the expanded state is on one side and in the contracted state on the other side of the membrane.

Another visualization of a carrier mechanism is the concept of a rotating carrier, either a molecule or a molecular segment which by rotating within the membrane carries substances from one side to the other (Danielli, 1954).

A mechanism based upon the rhythmically asymmetrical collapsing of an expanded protein lattice has been proposed by Bowyer (1954).

Numerous theories which could account for active transport have been put forward. Unfortunately, most of them have not been formulated in a way that allows testing them experimentally and choosing between them.

VI. Active Transport and Bioelectric Potentials

Interest in biopotentials was created in the middle of the last century, when Du Bois-Reymond (1848) demonstrated a potential difference across the isolated frog skin. The electric events following conduction of nerve impulses drew much attention during succeeding years and led to Bernstein's "membrane theory" (1902) which has been of great importance for the modern concept of nervous conduction. Although it was very well established that the ion species and the concentration of the ions in the media had a great influence on biopotentials, it was not realized until recently that ionic movements are essential for their generation. Thus Ussing (1948, 1949a) pointed out that the potential difference across the isolated frog skin is due to the active transport of sodium ions across the epithelium from the outside to the inside.

The development of microelectrodes that can be inserted into the interior of a cell to measure its potential with reference to another electrode in the external medium opened up a new way to study the activity of living cell membranes. The earlier microelectrodes allowed measurements of membrane potentials only in large cells like the giant axon of the squid (Hodgkin and Huxley, 1939, 1945; Curtis and Cole, 1940, 1942). But improvement of the technique (Ling and Gerard, 1949) made wider application possible and led to extensive studies of membrane potentials and their relation to ionic movements. In the course of these studies, many similarities have been revealed among the biological membranes that perform active ion transport.

A recent study of the nature of the frog skin potential (Koefoed-Johnsen and Ussing, 1956, 1958) may be used to illustrate a possible way in which a biopotential can develop.

Work by Ussing (1948, 1949a), Ussing and Zerahn (1951), and Linderholm (1952) clearly demonstrated that the spontaneous potential across the frog skin is the result of the active transport of sodium and the shunting by the anions, viz., chloride ions. Therefore, in order to study the electromotive force (E_{Na}) of the sodium transport mechanism itself, the shorting effects of the ions must be avoided. This can be done by substituting the virtually nonpenetrating sulfate ion for chloride, or by reducing the chloride permeability by treating the outside surface of the skin with Cu^{2+} at a concentration of $10^{-5} M$. In the absence of penetrating anions the outside of a frog skin (from *Rana temporaria*) behaves like a sodium electrode over a wide range of concentrations, while the inside behaves like a potassium electrode. In other words, the outward-facing membrane of the transporting epithelial cells seems to be selectively permeable towards Na^+ (and Li^+, cf. Zerahn, 1955), a selectivity which is comparable to that of the active nerve membrane.

while the inward-facing membrane is preferentially permeable towards potassium ions, i.e., similar to the resting nerve membrane. The permeability of the inner cell membrane towards free sodium ions (as distinguished from sodium passing through the " Na pump ") is extremely low. The epithelial cells presumably have a high potassium and a low sodium concentration. The sodium concentration of the cells is assumed to be kept low by active extrusion of sodium. Furthermore, the active sodium transport mechanism is assumed to be located at the inward-facing membrane and to be a carrier-linked, possibly a forced exchange of sodium from the cell against potassium from the inside bathing solution. This implies that the mechanism is an electrically neutral pump. A similar coupling between sodium and potassium seems to take place, as mentioned previously, in red cells (Harris and Maizels, 1951 ; Glynn, 1956), in muscle (Steinbach, 1952 ; Keynes, 1954), and in nerve (Hodgkin and Keynes, 1955a).

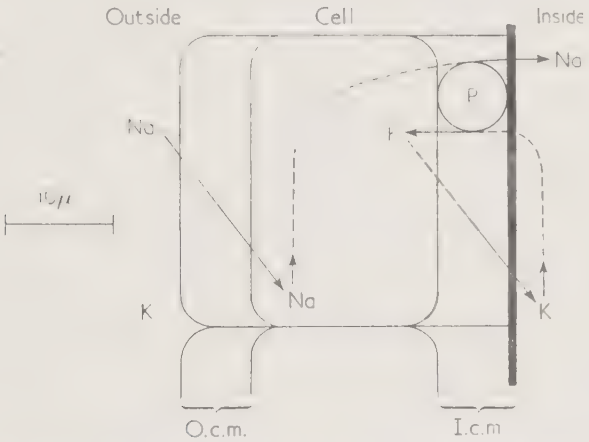

FIG. 2. A stratum germinativum cell with a model illustrating the hypothesis about the origin of the frog skin potential. *O.c.m.* = outer cell membrane, which is passively permeable to sodium. *I.c.m.* = inner cell membrane, which is passively permeable to potassium. *P* = pump.

The potential would then develop in the following way (Fig. 2). Sodium diffuses from the high sodium concentration outside across the outer cell membrane into the epithelial cell, thereby creating a diffusion potential E_o, the magnitude of which is given by the Nernst equation.

$$E_o = \frac{RT}{F} \ln \frac{\mathrm{Na}_{(o)}}{\mathrm{Na}_{(cell)}}$$

where R, T, F have the conventional meaning, $\mathrm{Na}_{(o)}$ is the sodium concentration in the outside bathing solution, and $\mathrm{Na}_{(cell)}$ is the sodium concentration in the cell. Sodium is then actively extruded by exchange against potassium from the inside bathing solution, and potassium from the cell diffuses back across the inner border into the inside solution and thereby creates another diffusion potential $E_{(i)}$. The magnitude of this potential is determined by the high cellular potassium and the low concentration of potassium in the inside bathing solution. Again, according to Nernst,

$$E_i = \frac{RT}{F} \ln \frac{K_{(cell)}}{K_{(i)}}$$

The total potential across the skin is then given by $E = E_o + E_i$. It will be noticed that potassium moves in a closed circuit, giving rise to no net gain or loss, and the active sodium-potassium exchange appears as an active sodium transport. The maximum potential E is reached only when the anion permeability is practically zero, and no current is drawn from the skin. It represents the electromotive force of the active sodium transport E_{Na} defined by Ussing and Zerahn (1951). R_{Na}, defined in the same paper, is then composed of a sodium resistance and a potassium resistance in series. In the general case, when the movements of anions have to be taken into account, the potential may be approximated by an expression similar to that which has been used by Hodgkin and Katz (1949) for the membrane potential of the squid axon.

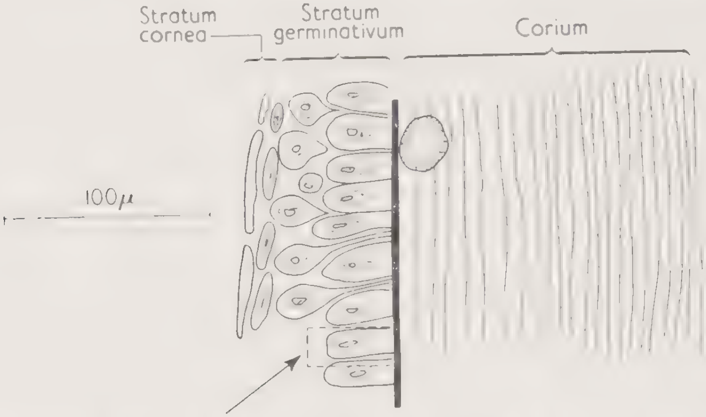

FIG. 3. A schematic drawing of a section through the frog skin showing the different layers. The arrow points to the part of the section which is shown in Fig. 2.

Potential measurements by means of micropuncture have been performed (Engbæk and Hoshiko, 1957) and have shown the existence of the two predicted potential steps in skins bathed in sulfate Ringer.

It would be natural to identify the potassium-selective membrane with the inward-facing membrane of the stratum germinativum, and the sodium-selective membrane with the outward-facing membrane of the same cells, as these cells seem to form the only continuous layer of living cells in the epithelium (Fig. 3).

Active transport of sodium seems to be the source of many biopotentials, for example, the resting potentials of muscle (Dean 1941), nerve (Hodgkin and Keynes, 1955a), sheep rumen (Dobson, 1955), and kidney tubule (Solomon, 1957). Since the manuscript went into print some further measurements of potentials in kidney tubules have been reported [Giebisch (1958) and Solomon (1959)]. In some plant cells, *Halicystis ovalis* (Blount, 1957) and *Nitellopsis* (MacRobbie and Dainty, 1958) the available evidence seems to be compatible with the idea that the potential is the result of an active transport of chloride as well as of sodium.

In the ileum of the rat both active chloride and active sodium transport appear responsible for the potential (Curran and Solomon, 1957), while in the colon of the toad and in the cecum of the guinea pig only sodium transport is operative (Ussing and Andersen, 1956). The potential across the gastric mucosa is generated by active transport of chloride (Hogben, 1951, 1955), and the same seems to be true in the sublingual gland (Lundberg, 1957a, b). During adrenaline stimulation of the isolated frog skin, an active secretion of chloride ions (presumably from the skin glands) contributes to the potential across the skin (Koefoed-Johnsen *et al.*, 1952b).

VII. Stimulating and Inhibiting Agents

Experiments made during anoxia and under the influence of different metabolic poisons have clearly demonstrated that active transport of ions depends on metabolism, in some cases aerobic and in others, anaerobic. Inhibition studies made during anoxia or by means of cyanide have shown that active ion transport in most tissues depends on respiration. An exception is frog muscle which is insensitive to cyanide (Keynes and Maisel, 1954), while mammalian muscle, for example rat diaphragm, is oxygen dependent (Creese, 1954). Mammalian red cells do not respire and therefore depend on glycolysis for their ion transport (Maizels, 1951), while most nucleated red cells (reptiles, birds) require aerobic conditions (Maizels, 1954). Duck erythrocytes seem to occupy an intermediate position, as they can use energy from either respiration or glycolysis and transport even better during anoxia (Tosteson and Robertson, 1956).

Studies of the effects of different metabolic inhibitors (or stimulating substances) have been carried out with the purpose of getting information about the nature of the biochemical reactions which drive ion transport. Dinitrophenol (which is known to interfere with the formation of high-energy phosphate groups in respiration) has a very marked inhibitory effect on active ion movements in several tissues, for example, gastric mucosa (Davies, 1951), frog skin (Fuhrman, 1952), and some plant cells (Robertson *et al.*, 1951 ; Scott and Hayward, 1954). In giant nerve fibers, dinitrophenol inhibits the active recovery processes, but has no effect on the generation of the spike, during which ions move downhill along their gradients (Hodgkin and Keynes, 1955a). The inhibiting effect of dinitrophenol is strong evidence for associating high-energy phosphate groups with active transport. The role of glycolysis during anaerobic ion transport—where such transport is possible, as in human red blood cells (Glynn, 1956)—seems also to be the provision of adenosine triphosphate, since addition of arsenate [which prevents ATP formation by replacing orthophosphate in the oxidation of glyceraldehyde-1-phosphate (Needham and Pillai, 1937 ; Warburg and Christian, 1939)] stops K accumulation, but not lactic acid formation.

Further evidence for dephosphorylation of ATP as the source of energy for the " Na pump " is given by Caldwell (1956) who examined the effect of cyanide and dinitrophenol on the phosphate esters of the squid nerve fiber. There are three main forms of phosphate in the nerve fiber : inorganic, ATP, and arginine phosphate. By applying cyanide or dinitrophenol in necessary concentrations, the Na efflux and the concentrations of ATP and arginine phosphate were reduced, while the inorganic phosphate fraction was increased. In analogy with the events in muscle, arginine phosphate acts by replenishing the ATP (Caldwell, 1957).

Attempts to promote ion transport by addition of ATP have generally not been successful, probably because ATP cannot penetrate cell walls. However, by injecting ATP into a cyanide-poisoned nerve fiber, Caldwell and Keynes (1957) succeeded in partially restoring the sodium efflux. The amount of sodium extruded was roughly proportional to the amount of ATP injected. Further discussion on the role of energy rich phosphate compounds in active transport has recently been given by Caldwell (1959).

If ion transport is generated by high-energy phosphate groups derived from glycolysis or aerobic metabolism, then any agent which stimulates or inhibits metabolism will influence ion transport, provided the substances in question can penetrate the cell. Thus addition of an intermediary product from the energy-yielding cycle might stimulate ion transport, if the formation of that product is the rate-limiting process in the metabolism of the tissue in question. Likewise, metabolic poisons like NaN_3, CO, and malonate will inhibit transport dependent on aerobic metabolism, while iodoacetic acid and fluoride inhibit ion transport dependent on glycolysis.

The known stimulating agents affect either the general metabolism or the permeability of the cell. So far no agent has been reported which directly stimulates the sodium pump.

Substances affecting cell permeability include various lytic agents (Ponder, 1948) and salts of heavy metals. Traces of copper strikingly lengthen the time required for hemolysis in 0.3 *M* glycerol (Jacobs and Corson, 1934). Tannic acid decreases the normal permeability of the erythrocyte to certain anions (Cl^-; Jacobs *et al.*, 1943). Pb salts cause a rapid net loss of potassium from human red cells (Henriques and Ørskov, 1936). In the isolated frog skin, Cu and Ag salts applied to the outside solution increase the potential across the skin by decreasing the chloride permeability of the outward-facing membrane of the epithelial cells (Ussing, 1949b; Koefoed-Johnsen and Ussing, 1958). Consequently they have no effect on the short-circuit current. When applied to the inside bathing solution, Cu and Ag salts increase both the potential and the short-circuit current (unpublished observations), presumably by increasing the K permeability of the inward-facing membrane of the epithelial cell layer.

Hormones from the posterior lobe of the pituitary gland, when administered to the inside bathing solution, increase the potential across the skin (Fuhrman and Ussing, 1951), the short-circuit current (Ussing and Zerahn, 1951), and the flow of water through the skin (Koefoed-Johnsen and Ussing, 1953). Both the oxytocic and the pressor fractions are effective. The effect is interpreted as an increase in the pore size of the outward-facing membranes of the epithelial cells.

If the CO_2 content is raised to 5% in the air mixture used for mixing and aerating the solutions bathing the frog skin, both the potential (Ussing, 1949a) and the short-circuit current are decreased (Ussing and Zerahn, 1951). The effect seems to be a decreased K permeability of the inner epithelial cell membrane, as the inside does not exhibit the K-electrode-like behavior during CO_2 treatment (Koefoed-Johnsen and Ussing, 1958).

Among substances inhibiting ion transport, the cardiac glycosides with a lactone ring seem to represent a special group. So far they are the only substances reported to act directly on the carrier mechanism. Thus strophanthin has been shown to inhibit ion transport in erythrocytes (Schatzmann, 1953; Joyce and Weatherall, 1955; Kahn and Acheson, 1955; Glynn, 1955, 1957; Solomon *et al.*, 1956a, b), in frog muscle (Johnson, 1956), in frog skin (Koefoed-Johnsen, 1957), and in squid giant axon (Caldwell and Keynes, 1957). Schatzmann demonstrated that strophanthin in concentrations which inhibit ion transport does not have any effect on oxygen consumption or glycolysis of red cells. The effect thus seems to be directly on the transport mechanism, and not on the energy supply of the pump.

The potato root eelworm hatching factor contains a lactone ring and has an effect similar to strophanthin (Ellenby and Gilbert, 1957).

Some dinoflagellates are known to make very powerful toxins. Abbott and Balantine (1957) tried the effect of a toxin from *Gymnodium* on several membrane potentials and concluded that the toxin acts by depolarizing the membranes. When administered to the inside of the frog skin, the toxin not only reduced the potential, but actually reversed it. This effect could be interpreted as an effect on the permeability of the inward-facing membrane. If the permeability of this membrane to sodium were increased to a point where it exceeded its permeability to potassium a reversal of the potential might be expected.

In a recent review Shanes (1958) has discussed the electrochemical aspects of physiological and pharmacological action in excitable cells.

VIII. Interrelations of Metabolism and Active Transport

In an active transport process work is done at the expense of energy derived from metabolism. Since nothing is known about how energy is transferred to an actively transported ion, most metabolic studies of transferring processes are concerned with comparison of the transport work with total energy production of the transporting tissue. Two questions are of main interest in the relation of active transport to metabolism, namely, how big a fraction of the metabolic energy do the cells have to spend on active transport of ions, and what is the maximal efficiency of active transport processes. The first of these problems can be dealt with more easily.

Estimates of the minimum energy requirements of active transport will often be invalidated by the fact that exchange diffusion processes may participate in the movements to an unknown extent. Thus the free energy requirements would be zero if all ion transfer in the tissue were brought about by means of exchange diffusion.

The work required to transport one mole of ion can conveniently be considered to consist of three components :

(*a*) the work required to overcome the concentration gradient,

$$W_1 = RT \ln (c_{(\mathrm{i})}/c_{(\mathrm{o})})$$

(*b*) the work to overcome the potential gradient,

$$W_2 = F (\psi_{(\mathrm{i})} - \psi_{(\mathrm{o})})$$

(*c*) and the work to overcome the internal resistance,

$$W_3 = RT \ln (M_{\mathrm{in}}/M_{\mathrm{out}})$$

Therefore the total work W is

(*d*) $W = W_1 + W_2 + W_3 = RT \ln (c_{(\mathrm{i})}/c_{(\mathrm{o})}) + F (\psi_{(\mathrm{i})} - \psi_{(\mathrm{o})}) + RT (M_{\mathrm{in}}/M_{\mathrm{out}})$

and can be expressed in calories by inserting 1 joule = 0.239 cal. The ratio $(W \times 0.239 \times 100)/(O_2 \times \text{caloric value})$ is called the thermodynamic or energetic efficiency.

For human red cells, the minimum energy requirements for the influx of potassium and the efflux of sodium in the steady state have been calculated by Maizels (1951) and Solomon (1952). From (*d*) it is seen that the flux ratio term in this case is zero, as influx and efflux are equal in the steady state. The potential can be estimated from the distribution of chloride ions, as this ion is assumed to be in thermodynamic equilibrium. The work was found (Maizels, 1951 ; Solomon, 1952) to be about 9 cal per liter of cells per hour. The energy available from the anaerobic metabolism of 1.5 millimoles of glucose, consumed in the same period, amounts to 72 cal, and the energetic efficiency, consequently, to about 12%. The transfer of sodium and potassium thus requires but a small percentage of the total energy released by glucose consumption.

Estimations of energetic efficiencies based on total oxygen consumption are not likely to give correct values as most probably only a small fraction of the oxygen is used for transport purposes. In some instances it is possible to get a determination of the " nontransport " metabolism of a tissue and, by subtracting this part from the " transport " metabolism, to get a net oxygen consumption for the ion transport. If this procedure is not feasible, it may sometimes be possible to stimulate ion transport by some agent and to use the increments in transport and oxygen consumption for calculating the efficiency. By using this procedure Zerahn (1956) found that the ratio between the rate of active transport and the net oxygen consumption in the isolated frog skin was practically constant and independent of the transport rate. This means that there is a stoichiometrical relationship between active ion transport and the metabolic rate.

The energy required for the formation of the gastric secretion has been the subject of considerable interest (see for example, Conway, 1953b). The work required for the formation of one liter of gastric secretion can be calculated from (*d*) for every species of ion transported. The equation may be reduced to the sum $W = RT \ln (c_s/c_p)$, where c_s is the concentration of the individual ions in the secretion and c_p the concentration in plasma. Because the internal resistance probably is very low, both the flux ratio term and the potential term can be disregarded, as electroneutrality has to be maintained. Furthermore, the secretion is isosmotic with plasma, so that the water term is zero. The formation of 1 mole of gastric acid takes 10,000 cal in mammals, where the concentration of HCl is $0.15N$ (Gray, 1943), but only about 8800 cal in the frog (Crane and Davies, 1951) as the frog gastric acid is weaker, $0.12N$.

These energy requirements can now be compared with the oxygen consumption.

Even more interesting than the relation between the minimum free energy needed for secretion and the maximum free energy available from metabolism is the maximum number of moles of HCl secreted per mole of oxygen required for the secretory process. The gastric mucosa of *Rana temporaria* does not secrete spontaneously during winter, but only after histamine stimulation (Crane and Davies, 1951). In such frogs Crane and Davies (1951) found that the ratio between the extra oxygen consumption and HCl formed was as high as 13 in a few cases. The energy released by one mole of oxygen during oxidation of carbohydrate is 116,000 cal, so that if conversion is 100% efficient, the formation of a maximum of 13 moles of HCl would be possible per mole of oxygen. If, however, the energy for secretion is furnished by ATP (which is probable, as dinitrophenol inhibits acid formation), and if the most recent value for the energy of the terminal bond of ATP (6000 cal, Podolfsky and Morales, 1956) can be used under these conditions, then it is hard to conceive how, at best, more than 6 moles of HCl can be formed per mole of oxygen, as 6 moles of ATP are formed per mole of oxygen. There is, of course, the possibility, discussed by Crane and Davies (1951), that part of the unstimulated respiration can be used for secreting purposes. Another possibility is that energy from glycolysis contributes to acid formation (Davenport and Chavré, 1952). However, the fact that more than four moles of HCl are formed per mole of oxygen rules out the possibility of explaining ion transfer on the basis of the electron-transferring theories.

Some studies on the relationship between sodium transport and oxygen consumption have been made on the isolated frog skin (Zerahn, 1956, 1958 ; Leaf and Renshaw, 1957). Early attempts to estimate the work done by the frog skin were made by Stapp (1947), who compared the oxygen consumption with the current drawn from a (partially) short-circuited skin, the isolated frog skin being considered as an electrical battery. The work, then, which such a battery can perform is measured by the maximum current strength delivered when the outer resistance is zero. Stapp calculated the result in per cent coulomb efficiency, which he defined as

$$\frac{\text{coulombs generated} \times 100}{\text{coulombic equivalents of } O_2}$$

Zerahn (1956) compared the rate of active sodium transport and the concomitant oxygen consumption of the isolated frog skin under different conditions. When the short-circuit current (which equals the active sodium transport) and the total oxygen consumption were compared, coulomb efficiencies as high as 200% were obtained. By subtracting the "nontransport" metabolism (when the outside bathing medium is distilled water or Ringer solution in which Mg or choline have been substituted for Na) from the total metabolism and calculating the coulomb efficiency on the basis of this net oxygen consumption, Zerahn found values of 400–500%.

If ion transport were coupled directly to the reduction of oxygen, not more than 4 faradays per mole of O_2 could be obtained (=100% coulomb efficiency). The high coulomb efficiencies found by Zerahn (1956) and Leaf and Renshaw (1957) exclude the possibility that electron transfer processes can be responsible for the active ion transport in the isolated frog skin.

The work done in the short-circuited skin with Ringer solution on both sides is given by $W = RT \ln(M_{\text{in}}/M_{\text{out}})$, since the potential and the concentration gradient are zero and only the internal resistance term is left. The flux ratio varies from 10–100 in different skins with Ringer solution on both sides, and W varies from 1340–2680 cal. With a net oxygen consumption of $\frac{1}{4}$–$\frac{1}{5}$ equivalent per equivalent of transported sodium, the thermodynamic efficiency varies from 20–50%. In terms of ATP, this means that 3–4 equivalents of sodium are transported per mole of ATP, and thus roughly 2000 cal is available for each equivalent of sodium, a number that does not conflict with the given values of 1340–2640.

The high ratio of transported equivalents of Na to equivalents of O_2 found in the frog skin persists under various conditions, whether the skin transports against a concentration or potential gradient, or whether the rate of transport is increased by stimulation from pituitary hormone (which reduces the internal resistance). The implication of these findings seems to be that the rate of active transport determines the rate of oxygen consumption, probably by way of its drain on the ATP supply.

Lundegårdh and Burström (1933) discovered an analogous stoichiometrical relationship for the ion uptake by wheat roots, where the oxygen consumption increased in proportion to the rate of ion uptake. Lundegårdh (1945) described the reaction as "anion respiration," assuming that one anion was taken up for each electron carried through the cytochrome system. These results have recently been reinterpreted by Chance and Williams (1956) in view of their own experiments on the effect of the ADP level on the electron transport chain of isolated mitochondria and of whole yeast and ascites tumor cells. According to Chance and Williams (1956), the "anion respiration" is then an active transport process requiring energy from a reserve of ATP. They assume that ADP diffuses to the mitochondria of the root where it activates the respiratory chain, as the concentration of ADP is rate-limiting.

Only a few experiments relating ion transport and metabolic processes have been reported, and further experiments along this line are essential for understanding transport processes. The ratio of ions transported to O_2 consumed is of particular interest in this respect. A knowledge of the upper limit of this ratio is important in the choice between the various model systems which have been proposed to explain active transport.

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CHAPTER 7

Body Fluid Dynamics

J. R. ROBINSON

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I. Scope of Chapter

A. Body Fluids

About two-thirds of the mammalian body consists of water containing a diversity of organic and mineral substances in solution. The solutes, however, are not uniformly distributed through a single homogeneous phase ; a number of subdivisions of the body water can be distinguished by their chemical composition or by their anatomical location and are spoken of in the plural as " body fluids." The body fluids are in fact all liquids. Gases are also fluid, but the only gases " in " the mammalian body are not truly inside it but in cavities, such as the alimentary tract and the organs of external respiration, which are surrounded by the body but continuous with the external environment.

B. Dynamics

Dynamics is a branch of mechanics devoted to the study of systems in motion, with special regard to forces as the cause of motion (Maxwell, 1877). Hence dynamics is concerned with transformations of energy in systems which are not in equilibrium, not merely with rates of change (which are dealt with in kinetics), nor with equilibrium conditions. This chapter attempts to present a descriptive outline of the fundamental principles of exchanges between the body fluids, with emphasis upon their dependence upon various forms of energy.

It will be convenient first to mention the biological importance of water and electrolytes and to give a preliminary outline of the body fluids.

II. The Fluid Background of Life

A. Water

Life as we know it exists within the rather narrow range of what are called " ordinary " temperatures. We begin to appreciate the vital importance of water when we reflect that this range lies between the freezing points of dilute aqueous solutions and the temperatures at which wet proteins are rapidly denatured. Henderson's classical account (1913) of the fitness of water to become the physical substratum of the living world has never been superseded, although we may now be better able to interpret the singular fitness of water in terms of its molecular structure. The two O-H bonds enclose an angle of about 105°, and the greater share which oxygen takes of the two electrons held in common with hydrogen gives the molecules a considerable dipole moment as well as a structure which is extremely favorable for linking together by hydrogen bonds. Single molecules hardly exist except in the vapor, and the liquid is strongly associated, so that of all the simple hydrides, water has the highest melting and boiling point and the greatest heat of vaporization (Moeller, 1952). The angle between the two O-H bonds is close to the tetrahedral angle (109° 28′), and it now seems agreed (Robinson and Stokes, 1955) that a tetrahedrally coordinated structure resembling that of ice persists in the liquid, at least over short distances and for intervals of time which may be only of the order of 10^{-12} sec but are long enough for the structure to be revealed by interaction with X-rays. The implied orientation of molecular dipoles in the liquid is the basis for the almost uniquely high dielectric constant which, more than any other single property, contains the secret of water's fitness to bring forth and sustain life. This high dielectric constant, which reduces electrostatic forces about eightyfold, makes water a supremely good solvent for ions ; the unique solvent power of water acting through geological ages upon the earth's crust has leached out of hard rocks and softer soils the mineral elements which are now accumulated as essential constituents in the bodies of living plants and animals.

B. Ions

Simple ions are so small in relation to their charge that enormous electrostatic fields, of the order of millions of volts per centimeter, exist at the boundaries between them and the surrounding medium. Consequently the dipolar water molecules immediately surrounding ions in aqueous solution are electrostatically bound. The energy exchanged in this process of hydration plays an important part in releasing ions from their lattice positions when solid electrolytes dissolve and so contributes to the efficacy of water as an " ionizing " solvent. Beyond the tightly bound " primary " shell of water molecules there is a rather diffuse cloud of loosely bound molecules whose adherence tails off with increasing distance as the electrostatic potential energy diminishes and ultimately becomes comparable with the energy of thermal agitation. The degree of hydration of an ion can therefore hardly be defined precisely, and Bockris (1949) pointed out that several methods for determining the hydration number of sodium gave figures ranging from 1 to over 700 water molecules per sodium ion, although methods which might be expected to measure " primary " hydration gave more concordant results. Robinson and Stokes (1955) discussed the evidence which suggests that a nonpolar molecule dissolved in water is surrounded by a region in which the structure is more orderly than in the pure liquid remote from dissolved particles, whereas the structure in the region just outside the primary hydration shell round an ion is more disorderly. In consequence of this " structure-breaking effect " it may still be justifiable to regard a hydrated ion as a particle with a reasonably well-defined size (cf. Conway, 1957).

Hydrated ions are several times larger in diameter than the corresponding naked ones and are bounded by correspondingly weaker electrostatic fields. Not only does water bring electrolytes into solution ; it also clothes the ions with water molecules which smooth the transition between ions and solvent and moderate their properties in ways of great importance in biology. Life is maintained by energy released in the cells by chemical reactions in aqueous solution ; these reactions can only proceed quickly enough at " ordinary " temperatures because they are catalyzed by enzymes, the activity of which often depends upon appropriate concentrations of particular ions (cf. Vol. I, Chapter 16). The maintenance of concentrations in special regions requires not only a supply of energy, but also selectively permeable membranes to restrict free diffusion; and here again ions are important because the permeability of many biological membranes depends upon the ionic pattern of the fluids which bathe them.

III. The Body Fluids

The subdivision of the body water into a number of more or less distinct " body fluids " rests upon several kinds of evidence.

A. Anatomical Subdivision

Gross anatomical observation of the body reveals systems of tubes and cavities ; histological methods further distinguish cells and spaces outside them which are believed to be occupied during life by fluids which form a hierarchy as regards their accessibility to investigation and modification by agencies outside the organism. Most accessible of all is the blood plasma. Then come fluids which can be reached from the plasma by crossing only capillary membranes ; these include the interstitial fluid, whether free or caught in the meshes of a connective tissue gel, the lymph drained from the tissue spaces into lymphatics, and transudates in some serous cavities. Intracellular fluids are accessible through cell membranes whose permeability is more restricted than that of capillaries. There is an innermost group of fluids like the cerebrospinal and ocular fluids and fluid in the synovial sacs which, although they may be easily sampled, are less accessible than intracellular fluids in the sense that they can only be reached from the blood by traversing, in addition to capillary membranes and interstitial fluid, two layers of cell membrane and the intervening cytoplasm of the complete layers of cells which enclose and secrete them. These are well designated " transcellular fluids ", although that term as originally defined by Moore (1948) included the fluid in the intestinal tract and the lumina of glands and their ducts, including the kidney and urinary tract. Although these fluids are extracellular, being outside cells, and transcellular, having been passed through cells, they are not truly body fluids because, although surrounded by parts of the body, they are not really inside it but occupy diverticula of the environment projecting into the body.

Edelman *et al.* (1952) pointed out that these extracorporeal transcellular fluids may become an important source of error because test substances which are assumed to be inside the body may be diverted into them.

B. Chemical Subdivision

Analysis of its various tissues and fluids reveals that the body is no more homogeneous chemically than anatomically. The plasma contains sodium and chloride as its principal mineral solutes, and the less accessible extracellular fluids are presumed to differ only in ways connected with their smaller protein content. The earliest analyses showed that despite the abundance of sodium and the paucity of potassium in these fluids, most tissues contained more potassium than sodium, so that the cells must be rich in potassium. The fact that the chloride in tissues was often accompanied by sodium in the same proportion as in the plasma led to the conception of a population of cells rich in potassium, permeated by more or less of an interstitial fluid which contained, like the plasma, mainly sodium and chloride with only a little potassium (Hastings, 1940). Not all differences between the amounts of sodium and chloride in different tissues can be due to differences in the proportions of cells and the sodium-rich fluid outside them. Manery and Hastings (1939) showed that if the sodium and chloride of rabbit gastric mucosa were present exclusively outside cells, then each kilogram of the tissue would need 500 ml of extracellular fluid to accommodate its chloride, but only 200 ml to accommodate its sodium. This discrepancy is most naturally explained by the presence of an unusually high concentration of chloride in the oxyntic cells which secrete hydrochloric acid. One kilogram of kidney tissue contained as much sodium and chloride as 500 ml of extracellular fluid, but this may be related to the fact that the tubular epithelial cells reabsorb, on the average, about one-half of their own volume each minute of the typical extracellular fluid which they continually secrete into the blood, and presumably contain sodium and chloride in transit, so that by no means all the sodium and chloride in renal tissue can be extracellular.

Tissue analysis is inevitably a destructive procedure which cannot readily be repeated in the same animal. The information derived from it is often out of date in the sense that it is not usually available until some time after the death of the animal and must be interpreted retrospectively. This is a more serious drawback in clinical investigation than in experiments with small and expendable animals. Moreover, the constituents found by the analysis of tissues cannot be assigned to extracellular and intracellular positions without assuming the distribution of one of them. The practical alternatives are to assume that all chloride or sodium is extracellular or else to determine the volume of extracellular fluid in the sample by an independent method.

C. " Compartmental Analysis "

1. *Analysis by Distribution at Equilibrium*

The volume of a homogeneous mass of liquid may be calculated from the concentration attained after a known amount of solute has been dissolved in it and evenly mixed. Since this often amounts to determining the extent to which a measured volume of a fairly strong solution of the test substance has been diluted to give the final concentration, such methods are known as " dilution methods ". They may be conveniently applied to living animals because substances introduced into the circulating blood soon become mixed and are ultimately distributed throughout those parts of the body to which they have access.

The ratio of the amount of test substance remaining in the body to the concentration in plasma water after equilibrium (or, in case the test substance is already present in the body as a normal constituent, the ratio of the additional amount remaining in the body to the increase in the concentration in plasma water) is known as the " apparent volume of distribution " of the test substance, or, more briefly, as its " space " (e.g. inulin space, urea space, etc.). It is usually calculated in liters and often expressed as a percentage of the body weight in kilograms. It is the volume of a solution, of the same concentration as the plasma, which would contain the amount of test substance in the body. It is called the " apparent " volume of distribution because a substance which was accumulated anywhere in higher concentration than in the plasma water would possess a calculated volume of distribution greater than the volume actually occupied by it. The working hypothesis that the test substance is present *in uniform concentration* throughout the fraction of the body water which is accessible to it is generally unverified.

Suitable substances for investigating the body water by dilution methods should be nontoxic and free from pharmacological activity, capable of being accurately estimated in concentrations too small to cause osmotic disturbance of the body fluids, and also physiologically inert, for otherwise they would be more likely to be accumulated in cells or destroyed by metabolism. Details of techniques, with means of allowing for excretion and for ensuring equilibrium, do not affect the principle of the method, and are not considered here. An excellent review of available methods will be found in Elkinton and Danowski (1955, Chapter 3).

It is a criterion of the homogeneity of a mass of liquid that its volume determined by dilution methods should be independent of the test substance employed. When dilution methods are applied to animals, different solutes have different apparent volumes of distribution, so that the body water is not a single homogeneous phase, but behaves as though subdivided into compartments by partitions which some solutes can cross more easily than others. The use of substances which have different apparent volumes of distribution to partition the body water into chemical subdivisions is a simple form of compartmental analysis. It has the advantage that it is applicable to the living animal and can be used in the clinical investigation of patients.

The apparent volumes of distribution of solutes in the mammalian body fall into three groups as regards order of magnitude. Relatively indiffusible colloids and substances which are bound to plasma proteins are confined in man, at least for a few minutes, to volumes of between 3 and 4 liters. Readily diffusible substances like urea and antipyrine come to equilibrium in a few hours in volumes of about 35–45 liters, or 50–70% of the body weight. A third group of substances like sucrose, inulin, thiocyanate, thiosulfate, sulfate rather consistently give intermediate values of 12–17 liters, or 16–25% of the body weight. It is not difficult to identify the largest and smallest of the compartments delimited in this way. Three to four liters is an acceptable figure for the volume of the blood plasma and agrees with the plasma volume determined by other methods. That the largest volume is that of all the water in the body is confirmed by its agreement with the amount found after death by drying the carcass. It is further confirmed by the fact that heavy and tritiated water are diluted in a similar volume. However, this overestimates the total volume of body water in so far as these substances exchange with hydrogen in compounds other than water and also enter the transcellular fluids, including those strictly outside the body. The intermediate values (12–17 liters in man) may be crudely identified with the total volume of extracellular fluid, but the actual figures vary because the solutes differ in the completeness of their penetration throughout the extracellular fluids and also in the extent to which they spill over into other compartments. Inulin, which gives the lowest volumes of distribution in this group, having a molecular weight of about 5000 and asymmetric particles, is probably partially excluded from the gelatinous matrix of connective tissues. Chloride, on the other hand is more concentrated in tendon and connective tissue than in the free extracellular fluid, so that even if there were no chloride in cells, the apparent volume of distribution of chloride should lead to overestimation of the volume of extracellular fluid. Nichols *et al.* (1953) allowed for the imperfect penetration of inulin into connective tissues and for the preferential accumulation of chloride, and defined " connective tissue corrected " inulin and chloride spaces which differed far less than the apparent volumes of distribution of the two solutes. Elkinton and Danowski (1955) gave an excellent comparative account of the results obtained with different solutes and a complete list of references.

2. *Kinetic Analysis*

When recognizable isotopic variations of the common ions of the body became available, the sensitivity of methods for the estimation of the radioactive isotopes made it possible to apply dilution methods without the need to introduce foreign substances or to use such large quantities of test substances as to disturb the balance between the compartments osmotically. Besides allowing the volumes of distribution of normal body constituents to be measured, the use of radioactive isotopes provided wholly independent confirmation of the existence of more or less distinct fluid phases within the body. Instead of waiting for even mixing to be achieved, it became relatively easy to follow the time course of the distribution of a test substance through the body by plotting its concentration in the plasma against time. Distribution through a single homogeneous phase should yield a simple exponential function. In general a more complex curve was obtained which could be analyzed to yield a series of exponential functions with different time constants characterizing masses which equilibrated with the plasma at different rates (cf. Vol. I, Chapter 5). Thus Kaltreider and his collaborators (1941) found that Na^{24} was rapidly distributed in a presumably extracellular phase making up about one-quarter of the body weight, and then penetrated further very slowly. Manery and Bale (1941) killed rats and rabbits at increasing intervals after the intraperitoneal injection of Na^{24} and so showed that the rapid phase of distribution corresponded not only with entry into the extracellular fluid spaces of all the tissues, but also into the cells of some organs, such as the kidney and the gastric mucosa. The slower phase was identified with penetration into the central nervous system (including the cerebrospinal fluid) and the skeleton, which incidentally contains about half the sodium in the body (cf. Vol. I, Chapter 13 and Chapter 14).

Experiments in which equilibration was complete had led to a somewhat static conception of the fluid compartments, but these kinetic studies served to emphasize the rapidity with which exchanges are continually occurring. The constancy of their volume from animal to animal and from time to time seemed more remarkable in the light of these rapid exchanges and was shown to depend upon a rather precise balancing of the rates of movement in opposite directions across the boundaries between the compartments (cf. Robinson, 1953).

3. *The Relations between Anatomical and Chemical Compartments*

The use of isotopes of normal constituents of the body also made possible a new approach to the problem of how far sodium or chloride could be assumed to be extracellular, for the volumes of distribution of these ions could be compared with the best estimates of the volume of extracellular fluid made with foreign substances. Table 3–4 on p. 78 of Elkinton and Danowski's book (1955) shows that the sodium and chloride spaces are somewhat larger than those of sulfate and thiosulfate, which probably provide the best estimates of the volume of extracellular fluid. This alone is not enough to settle the matter because of the accumulation of sodium in cartilage and chloride in fibrous tissues and the exchange of a considerable part of the sodium in bone with sodium in the extracellular fluid. After allowance has been made for these irregularities it seems that the chemically demarcated compartments correspond only approximately with the anatomical extracellular and intracellular fluids. Just as extracellular fluids contain some potassium, so intracellular fluids contain some sodium and probably a smaller amount of chloride. But these quantities are small enough, and the cells which contain considerably more of the typically " extracellular " ions are few enough, to make it possible for many purposes to regard the body as composed of two main chemically distinct phases, one intracellular and rich in potassium and the other extracellular and rich in sodium and chloride, although the sodium in the cells and the potassium in the extracellular fluids cannot always be ignored. Further detailed information may be found in the review by Edelman and Leibman (1959).

D. Functional Interrelations

1. *The Body Fluids as Phases*

Although it is often convenient to refer to the various body fluids as " phases ", the word cannot be used rigorously as in physical chemistry. Phases, as strictly defined, must be homogeneous as well as physically distinct. Since living cells are always consuming and producing substances in solution, there is a continuous traffic inwards and outwards in response to gradients of concentration which must extend away from the membranes on both sides, and the fluids adjacent to cell membranes cannot be strictly homogeneous in composition. Only if all concentrations had been equalized would it be proper to speak of homogeneous phases in equilibrium ; and then the animal would be dead.

It is moreover a great oversimplification to think of two fluid phases only, extracellular and intracellular. The extracellular parts of the body do not form a single fluid phase, and they include structures such as the collagen fibers of tendons and connective tissues and crystallites of bone mineral, which are not fluid. While the plasma and interstitial fluids represent an extracellular phase which is tortuous, yet in the main continuous, the intracellular fluid is present as millions of tiny subunits, each more or less independent and isolated within a membrane of restricted permeability. The cell fluids may be crudely likened to the discontinuous dispersed phase of an emulsion of which the extracellular fluid constitutes the continuous dispersion medium. It follows that a molecule in solution can only pass from one cell to another by traversing two cell membranes and an intervening layer of extracellular fluid : and all exchanges between cells and the external environment must be conducted through the extracellular fluid as middleman.

2. *The Importance of Volume*

Each member of a colony of *Amoeba* is so tiny in relation to the pond in which it lives that it may be considered as a single cell in an unlimited fluid environment. Its needs for oxygen and foodstuffs and its output of waste products and heat are so small that its activities do not sensibly alter the composition of the water, and the pond can function as an unlimited source of supply and an unlimited sink for the disposal of waste products and heat. The cells of a mammal's body maintain a higher temperature than *Amoeba*, and their metabolic demands and their output of waste products and heat are correspondingly greater. Many of them have become highly specialized and have at the same time become exacting in their requirements and intolerant of more than trivial alterations in the composition or temperature of their fluid environment. This fluid environment is the extracellular fluid, Claude Bernard's " milieu intérieur ", which, from the point of view of the cells, is an external environment whose constant intensive properties provide an essential foundation upon which the cell can stabilize its true internal environment, the intracellular fluid in which the fundamental processes of life go on. Unlike the environment of *Amoeba*, mammalian extracellular fluid does not greatly exceed the volume of the cells it sustains : in man, for example, about 45 kg of cells are crowded into 15 liters of extracellular fluid. Special mechanisms have been developed to regulate the composition of the extracellular fluid in higher animals, and their study forms a large part of physiology. Respiration, digestion and absorption, and selective renal excretion are all directed to this end : but these mechanisms depend upon the circulation of the blood, which keeps the extracellular fluid stirred and smoothes local alterations in composition or temperature. The plasma is a protein-rich part of the extracellular fluid, enclosed within the blood vessels. Its volume tends to reflect changes in the total volume of extracellular fluid. And since the adequacy of the circulation depends upon the volume of circulating blood, it has become necessary for the body to control one extensive property, the volume of its extracellular fluids, as a step towards fixing the intensive properties upon whose constancy every living cell depends.

It has taken longer to appreciate the importance of volume than of composition, for it seemed that the brisker regulating mechanisms responded to alterations in concentration, and it used to be thought that the body was relatively indifferent to the volumes in its fluid compartments. But daily weighing shows that the volume of water in the body is regulated, albeit slowly, to within one or two percent, a nicety which compares favorably with the regulation of most intensive properties. A vast literature on the regulation of the fluid volumes of the body is now growing up. The topic lies beyond the scope of this chapter; it is reviewed from different aspects by Peters (1952), Robinson (1954), Elkinton and Danowski (1955), Smith (1957), and in Vol. I, Chapter 12.

E. A Note on Interphases

The interphases which separate the principal phases of the body water are more complex than the thin, unhomogeneous interfaces between the more rigorously defined phases of physicochemical systems (Guggenheim, 1933, 1957). They are extensive on account of the fine subdivision of the intracellular compartment. The surface area πd^2 of a sphere of diameter d divided by the volume $(1/6)\pi d^3$ amounts to $(6/d)$ cm^2 per cm^3; and the surface area $\pi d^2 l$ of a long cylinder of diameter d and length l, divided by its volume $(1/4)\pi d^2 l$ amounts to $(4/d)$ cm^2 per cm^3. It follows that in an organ composed of spherical cells 20μ in diameter, the surface area per unit volume is $6/(2 \times 10^{-3}) = 3000$ cm^2/cm^3 of cells. For mammalian muscle, composed of fibers averaging 50μ in diameter, the average surface area per gram of muscle cells is similarly of the order of $4/(5 \times 10^{-3}) = 800$ cm^2, assuming a specific gravity of unity. The interphase must include the cell membrane, and if this were of the order of 100 Å in thickness (Davson, 1951), its volume per gram of spherical cells 20μ in diameter would be about $3000 \times 100 \times 10^{-8} = 3 \times 10^{-3}$ cm^3, or about a third of one per cent of the tissue, an amount which is not negligible, but small in relation to its importance. For the interphases of the body include the permeability barriers and possibly some of the pumping mechanisms upon which the essential differences between the several body fluids depend, and they may contain the common mineral ions of the body in quite different concentrations from the fluids on either side. Danielli (1951) has suggested, for example, that hydrogen ions may be more concentrated and calcium ions much more concentrated in the region immediately in contact with a cell than in the free fluid. As in Donnan membrane distributions, the ratio of the concentrations in surface and in free fluid for a divalent ion is the square of that for monovalent ions, so that the concentrations of calcium and sodium in the interphase may differ far less than in extracellular fluids.

The interphases of the body are singularly inaccessible to sampling techniques and cannot readily be studied except in isolated cells or portions of cells. They have been mentioned because of their great importance in relation to their small contribution to the body weight and the little that is known about them.

IV. Forces and Mechanisms Concerned in Exchanges between Fluid Compartments

A. Hydrostatic Pressure

1. *General*

Differences in hydrostatic pressure may move fluids within the body in two different ways. They may cause movements of liquids in bulk in the direction of diminishing pressure through channels of which the cross sections are large compared with molecular dimensions. They may also cause differences in thermodynamic activity which modify exchanges by diffusion through channels of a molecular order of magnitude and modify exchange through nonporous partitions in which permeation occurs by solution in or chemical combination with the material of the partition.

2. *Bulk Flow*

In bulk flow all the molecules in a small element of volume have the same average velocity superposed upon their random thermal motion and move without any tendency for the constituents of a mixture to be separated. This is a form of movement without change of composition *within* phases, in contradistinction to movement *between* phases, which may be associated with changes in composition because different types of molecules generally cross boundaries between phases at different rates.

In the body, movements of liquids in bulk under the influence of gradients of hydrostatic pressure are almost confined to the vascular systems. The aorta functions as an elastic reservoir replenished intermittently by the heart and maintaining a pressure about 100 mm Hg above the surroundings of the body. Chemical energy released from high-energy compounds in the contracting myocardium is converted partly to potential energy in raising the pressure in the ventricles, partly into the kinetic energy of the moving blood ejected during systole, and stored between beats as potential energy in the stretched walls of the distended aorta and arterial tree.

Except in large vessels where irregularities in the walls or unusually rapid flow through segments where the cross section is changing may give rise to turbulence, the flow of blood is usually laminar and obeys what is generally known as Poiseuille's law, after the French physician who found in 1840 that the volume of blood flowing in unit time through a cylindrical tube was proportional to the pressure gradient and to the fourth power of the radius. (This is also sometimes known as the Hagen-Poiseuille law because a German engineer, Hagen, had independently published the same empirical law for flow through pipes in 1839 [see Prandtl, 1936; Moelwyn-Hughes, 1957]). Poiseuille's law is usually written in the form:

$$Q = \frac{\pi}{8\eta} r^4 \frac{p_1 - p_2}{l}$$

where p_1 and p_2 are the pressures at two points separated by a distance l, and r is the radius. The coefficient of viscosity η is defined by Newton's law of viscous flow, from which Poiseuille's law can be derived. Newton's law states that $F = A\eta(dv/dx)$, where F is the tangential dragging force between adjacent fluid layers of area A, and dv/dx is the gradient of linear velocity at right angles to the direction of flow. Thus the force per unit area is proportional to the velocity gradient, and the coefficient of viscosity is the constant of proportionality. Liquids for which η is in fact constant are known as "Newtonian." Frequently in colloidal systems η is not constant, but increases at low values of the velocity gradient. Such liquids are known as "non-Newtonian," their viscosity is said to be anomalous, and it may then be useful to regard η as the rate of dissipation of energy in unit volume of the liquid for unit value of the *square* of the velocity gradient (Robinson, 1939; for a short general account, see Alexander and Johnson, 1949). Plasma shows Newtonian behavior, with a viscosity about twice that of water. The suspended corpuscles roughly double the over-all viscosity at ordinary hematocrit ratios and also make whole blood non-Newtonian, so that it does not strictly obey Poiseuille's law in small tubes, and the increasing viscosity at low velocity gradients should make flow through the arterioles even more sensitive to their caliber than the fourth-power law would suggest. The viscosity of whole blood is strongly affected by the concentration of erythrocytes (Whittaker and Winton, 1933) and rises very steeply when the hematocrit exceeds 50%. This viscous anomaly is associated with a separation of corpuscles from plasma within the smaller vessels, the corpuscles tending to move as a central plug faster than the layer of plasma near the walls where the velocity gradient is greatest. This mode of flow leads to differences in hematocrit in different parts of the circulation, and arterioles branching out from the sides of small arteries tend to skim off the plasma (Krogh, 1922). Pappenheimer and Kinter (1956) have recently suggested that plasma skimming of this kind, together with the rapid increase in viscosity as the central core of blood becomes enriched in corpuscles, may account for some peculiarities of the circulation within the kidney. This stimulating suggestion has not yet been widely accepted (Bradley, 1957), and workers in Pitts' laboratory were unable to confirm that severe anemia grossly impaired the autoregulation of the glomerular filtration rate or of renal blood flow in dogs (Thompson *et al.*, 1957; Kessler *et al.*, 1957).

General accounts of hemodynamics and of the physiology of the circulation are to be found in standard texts, and we shall take for granted here the mechanisms which regulate the cardiac output and distribute it among the various tissues and organs according to their current needs. Bulk flow round the circulation keeps the contents of the intravascular compartment mixed. Exchanges with other compartments are practically restricted to the smallest vessels, whose walls are thin enough to permit exchanges with the fluid outside. These are just the vessels in which flow is least satisfactorily described by Poiseuille's law, and pressure becomes as important in determining exchanges across the wall as in overcoming resistance to flow ahead.

3. *Filtration*

A porous membrane may be considered as made up of an indefinite number of capillary channels which differ in length and shape but are all arranged in parallel between the two sides of the membrane. Provided that these channels are wide compared to molecular dimensions, the flow through each of them appears to be laminar and to obey Poiseuille's law. A Newtonian liquid therefore should flow through such a membrane at a rate which is inversely proportional to the coefficient of viscosity and directly proportional to the difference in pressure between the two sides and the area of membrane involved. This appears to be true for water if the pores are not smaller than about 40 Å in diameter (about 20 molecular diameters), but deviations from the behavior predicted from Poiseuille's law appear when the pores are smaller and become very important at diameters less than 20 Å (see detailed discussion by Pappenheimer, 1953).

Porous membranes whose pores are very large on a molecular scale are employed to separate solid particles from heterogeneous mixtures, and filtration is a special case of bulk flow in the sense that all molecules of the liquid phase move together without change in composition. Neglecting effects of temperature upon the size of the pores, the temperature coefficient of the rate of filtration should be that of the viscosity of the liquid ; but a change in the size of the pores without altering the number in a unit area should affect the rate of flow in proportion to the fourth power of the pore radius. If the term filtration is reserved for bulk flow through porous membranes, it will later become apparent that "filtration" in biological systems may often be a mixture of filtration, ultrafiltration, and diffusion.

B. Diffusion

1. *Free Diffusion*

Unlike the essentially orderly movement of the molecules of a fluid flowing in bulk, diffusion is a disorderly movement molecule by molecule. Free diffusion is a relatively rapid process which occurs in dilute gases where the mean free path is large, so that the diffusing molecules do not interact, but move independently of their neighbors without often meeting.

2. *Liquid Diffusion*

Strong mutual attractions pack the molecules closely together and restrict diffusion in liquids. Although thermal agitation is not quite limited to vibration about fixed lattice points as in crystals, molecules do not often jump distances comparable with their size, and can only do so if they acquire a sufficient excess of kinetic energy to overcome the attractions of their neighbors or else if the surrounding molecules happen to move aside and leave holes into which they can fit (cf. Davson and Danielli, 1943, Chapter 5). Diffusion is therefore much slower in liquids than in gases, but the ions or solute molecules in a dilute solution are surrounded by solvent molecules and are so far apart that they may be considered to move independently of each other like the molecules of gases. Such independent diffusion is often referred to as free diffusion in contradistinction to diffusion restricted by small pores or by interaction between diffusing particles.

3. *Net Transport by Diffusion*

Since the molecular jumps are all completely random, with no preferred direction, a definite fraction of the molecules from any plane layer in a liquid will jump in any specified direction, while an equal number jump in the opposite direction, and the thermal motions of the molecules do not lead to any movement of the liquid in bulk. If, however, the same fraction of the molecules of each species moves to the right and to the left from layers adjacent to a plane separating regions of different concentration in a solution more concentrated on the left and more dilute on the right, then more solute molecules will cross the plane from left to right and more solvent molecules from right to left than *vice versa*. The fact that the jumps are random in direction has the direct consequence that more molecules of any kind leave a region where there are more to jump than a region where there are fewer. This process must go on spontaneously until all macroscopic inequalities of concentration have been eliminated, after which the numbers of molecules moving in any pair of opposite directions become equal and the diffusion of molecules continues without leading to any net transfer of matter. In contrast to their movements during bulk flow, the net movements of solvent and of solute are in opposite directions, and net transport by diffusion cannot properly be referred to as a flow or streaming. The rate of transport of any species across a plane is spoken of as its "flux," and the rate of *net* transport is the difference between the fluxes in opposite directions (cf. Vol. I, Chapter 2 and Vol. I, Chapter 6).

Diffusion can be treated thermodynamically because entropy is a measure of the randomness of a configuration. The chaotic arrangement in a uniform solution represents a more probable configuration than a gradient of concentration, and the dispersal of a concentration gradient is associated with an increase in entropy, which is equivalent to a decrease in Gibbs free energy (the mark of a spontaneous process) because $\Delta F = \Delta H - T\Delta S$ in a system at constant temperature and pressure (Bray and White, 1957). In the absence of appreciable heat of dilution ($\Delta H = 0$), the decrease in free energy corresponding to the increase in entropy represents the sole driving force of the diffusion process. The concentration gradient is like a spring which is wound up; after it has run down there is no further net transport, and macroscopic inequalities do not arise in uniform solutions in the absence of external forces. The net transport of matter by diffusion is a passive movement towards equilibrium, and continuous transport can only occur if the inequalities in concentration are maintained as a steady state by some active process supplied with energy from outside the diffusing system.

The rate of *net* transport across a plane by diffusion is given by Fick's law as $dn/dt = -DA\,(dc/dx)$, which states that the number of molecules (or moles) crossing an area A at right angles to the X axis in unit time is equal to the product of the area, the gradient of concentration in molecules (or moles) per unit volume, and a constant D called the diffusion coefficient. The minus sign is needed because the direction of net transport is down the gradient of concentration. This equation was adapted from Fourier's equation for the flow of heat in a uniform conductor, and it assumes, what is not strictly true in liquids, that diffusion is a continuous process (cf. Vol. I, Chapter 2). Like Poiseuille's law, which can also be derived by considering flow as a continuous rather than as a molecular process, it begins to fail in systems approaching molecular dimensions. Even in macroscopic systems, better agreement with experiment is obtained if the chemical potential of the diffusing substance is employed in place of its concentration; in the case of ions, the electrochemical potential must be used to include the influence of electrostatic fields.

Fick's law gives the rate of net transport as the quantity of matter crossing a plane in unit time, *not* the speed of the moving particles or the rate at which a layer of given concentration moves (cf. Hitchcock, 1945). The diffusion of particles which are large enough to be seen under the microscope or ultramicroscope can be directly observed as a jerky random trot which is known as Brownian movement, after the English botanist who observed it in a suspension of pollen. Measurements of Brownian movement confirmed the prediction arrived at by several theoretical approaches (Moelwyn-Hughes, 1957) that the resultant displacement should increase in proportion to the square root of the time. Similarly, the time taken to approach steady-state concentrations in diffusion increases with the square of the distance from the source. Thus Jacobs (1935) pointed out that for a solute diffusing into pure solvent from the plane boundary of a solution maintained at a fixed composition the concentrations $1\,\mu$, $10\,\mu$, $100\,\mu$, and 1 mm from the boundary should reach 99 % of their steady state values in 0.000456, 0.0456 and 4.56 seconds, and 7.6 minutes, respectively. Diffusion is therefore an extremely rapid means of equalizing concentrations over small distances of the order of the size of living cells and their membranes, but an extremely slow one over large distances. Mixing in large masses of fluid depends more on convection or on eddies produced in turbulent flow, and it is important to note that convection currents are a special case of bulk flow in that solvent and solute move together. They accelerate mixing by subdividing the liquid into small enough regions for the actual equalization of concentration to occur rapidly by the fundamental process of diffusion. Detailed calculations of the kinetics of diffusion under different conditions are given by Jacobs (1935) and by Crank (1956).

Since the principle of equipartition of energy assigns the same amount of kinetic energy ($\frac{1}{2}kT$, where k is Boltzmann's constant and T the absolute temperature) to each degree of freedom of every molecule, diffusion coefficients might be expected to depend upon molecular weight. The simple inverse proportion to the square root of the molecular weight observed approximately in gases does not hold for diffusion in liquids, but molecules of different sizes do tend to be separated during diffusion, so that net transport by diffusion, unlike transport by bulk flow, may be associated with a change in composition. Ions reach a compromise ; light cations cannot outstrip heavier anions because of the enormous potential gradients which would result from the separation of charges (Guggenheim, 1957, p. 372).

4. *Restricted Diffusion and Permeability*

A porous membrane having pores large on a molecular scale should, if placed in a concentration gradient, reduce the cross section available for diffusion and possibly increase the distance, but diffusion should otherwise be unaffected. Membranes of sintered glass are in fact used in the experimental determination of coefficients of diffusion to eliminate convection currents without interfering with diffusion (cf. Hitchcock, 1945). Alteration in pore size should affect the rate of diffusion through a porous membrane in proportion to the available area (proportional to the square of the pore radius) in contrast with their effect on bulk flow, which, as has been mentioned, should vary with the fourth power of the radius of the pores.

The mechanism of permeation through a porous membrane depends very largely upon the relative sizes of the pores and of the liquid molecules. When pores are much larger than the largest solute molecules, both solutes and solvent are free to diffuse through them. Such large pores also permit bulk flow of the solution as a whole when there is a gradient of hydrostatic pressure to provide energy ; and this flow of the liquid in bulk must profoundly modify exchanges by diffusion, for fluxes in the direction of the stream will be augmented, and contrary fluxes diminished or even reversed. Solvent drag effects arising in this way from diffusion in a flowing liquid were considered by Ussing (1954) in relation to the movement of ions through porous membranes (cf. also Vol. I, Chapter 6).

With smaller pores, not much larger than the solute molecules, the effect of bulk flow becomes less overwhelming, but a new complication appears in that steric factors and, in the case of ions, surface charges on the material of the pores begin to interfere with free diffusion. Moreover, when solutes of several molecular sizes are present, the restrictions upon diffusion become selective, and the tendency for different molecular species to be separated is far greater than when diffusion takes place in free solution. The process has been vividly described as molecular sieving. Exchanges through pores of this size involve both bulk flow (when there is a gradient of hydrostatic pressure) and restricted diffusion, the relative contributions of each depending upon the sizes of the pores and the permeating molecules. According to Pappenheimer (1953), water may be expected to exchange almost entirely by hydrodynamic flow through pores over 20 Å in radius, but net diffusion becomes increasingly important as the pores get smaller. It is often convenient to focus attention upon the relative ease with which different substances cross a membrane by speaking of it as more or less permeable to them and by describing the rate of exchange of each substance by a permeability coefficient rather than by a modified diffusion coefficient. Since the path length through the membrane is usually indeterminate, the rate of transfer is often expressed in terms of the difference in concentration between the two sides, and the unknown path length is incorporated into the permeability constant.

Pores smaller than the solvent and the smallest solute molecules would not permit exchange by either bulk flow or diffusion, but penetration through pores may not be the only way across a membrane. Homogeneous (nonporous) membranes may be crossed by molecules which can dissolve in or combine with their substance, diffuse across in solution, and then re-enter the liquid phase at the other side. The concentration ratios between the bulk phase and the membrane at each boundary depend upon the partition coefficients between the membrane substance and the liquid at either side. These partition coefficients, together with the activation energy which may be necessary for a molecule to shed its hydration shell and pass from the aqueous phase into the membrane [and which may be revealed by a large temperature coefficient of permeability (see Danielli, 1954 ; Davson and Danielli 1943)] help to determine the concentration gradient within the membrane, and consequently the rate of diffusion across it. Such membranes allow permeation by a form of restricted diffusion with no provision whatever for bulk flow under gradients of hydrostatic pressure. There has been much argument whether solutes cross biological membranes through pores or by dissolving in the membrane. Opposite opinions in physiological controversies have often both been partly correct, and cell membranes may exhibit pores in a material which is not merely a barrier reducing the area available for exchange, but an additional channel for the diffusion of substances which are soluble in it or can combine with some carrier substance or mobile combining group in the membrane. On the vast topic of the permeability of natural membranes, see also Höber (1945), Davson (1951), and Harris (1956, 1957). An important comparison of the permeability of porous and " solubility " membranes was published by Kuhn (1951).

An unusually interesting special case of restricted diffusion is provided by exchange across a membrane which has pores larger than the solvent but smaller than all solute molecules; or across a nonporous membrane permeable only to the solvent.

5. *Osmosis*

If a membrane which is quite impermeable to solutes but permeable to the solvent is placed between a solution and the pure solvent, diffusion of solute into the solvent is stopped, but diffusion of solvent molecules into the solution is merely slowed by the restrictions imposed by the membrane. There is therefore a net transfer of solvent through the membrane into the solution, which is called osmosis. Osmosis is a special case of restricted diffusion of the solvent down its own gradient of activity, and this gradient is its driving force. Its thermodynamic explanation is that all solutes depress the activity of the solvent, and this depression is roughly proportional to the total concentration of solute or solutes.

In a system obeying Raoult's law, i.e., in which solute and solvent molecules do not interact, the activity of the solvent is proportional to its mole fraction, and its depression by solutes is proportional to the sum of all their mole fractions. Guggenheim (1933) pointed out that molar concentrations may be regarded as proportional to mole fractions in aqueous solutions up to about $0.5\,M$ with a loss of accuracy of the order of 1%, and it is a common practice to express total concentration in osmoles per liter. The osmolar concentration of a nonelectrolyte is the same as its molar concentration. A mole of NaCl provides twice as many dissolved particles as a mole of glucose or urea, and a mole of $CaCl_2$ provides three times as many. Hence the osmolar concentration, in osmoles per liter, is the sum of the molar concentrations of nonelectrolytes together with the sum of the molar concentrations of the electrolytes, each multiplied by the number of ions to which it gives rise. Although most biological solutions are sufficiently dilute for osmolar concentrations to provide a good approximation to the depression of the mole fraction of the solvent, these do not provide so good an approximation for the depression of the solvent activity, because most of the solutes are electrolytes and their ions interact with each other as well as with the solvent. Allowance may be made for departures from ideal behavior by using empirically determined osmotic coefficients such as are listed by Robinson and Stokes (1955) and Harris (1956), but the theory of solutions has not yet reached a stage at which it is possible to make accurate calculations of the activity of water in mammalian body fluids from a detailed knowledge of their chemical composition.

A membrane through which solvent but no solute molecules can diffuse is known as an " ideal " semipermeable membrane. Actual membranes are usually less discriminating and merely restrict the diffusion of different molecular species to different extents. If a membrane which solutes penetrate at different rates is placed between a solution and the pure solvent, there will be net movements of the solvent and the various solutes until all their concentrations (or activities) are equalized, when the activity of the solvent will be the same on both sides of the membrane, and net transfer of solvent (osmosis) will cease too. Both solutes and solvent will still continue to diffuse through the membrane in both directions, but since all pairs of opposite fluxes will be equal, there will be no *net* transport of any species. If this state of equilibrium is disturbed by adding solute to the solution on one side of the membrane, there will be a temporary net transfer of solvent to this side which will cease when all solutes including the new one have again become evenly distributed. Such " osmotic transients " are common in living systems, for biological membranes are not as a rule ideally semipermeable. Cell membranes may, however, exhibit something approaching ideal semipermeable behavior towards solutes to which they are in fact permeable when the passive diffusion of these solutes through them is balanced by active transport in the opposite direction. Differences in the concentrations of penetrating solutes may then be maintained as steady states ; and their effect upon the activity of water is the same as though the membrane was impermeable to them.

Instances are known of " anomalous " or " negative " osmosis, in which there is net movement of solvent through a membrane from a solution into the pure solvent at the same pressure. These are often examples of electroosmosis, wherein an electric field causes movement of the molecules of a liquid past a charged surface. The phenomenon is related to the streaming potential which may arise when a charged solid moves through a liquid (McInnes, 1939). The possible importance of electroosmosis as a means of transporting water has been considered in detail by Sollner (see Shedlovsky, 1955). Transport of water across a membrane against its activity gradient may also occur when different temperatures are maintained at the two sides of the membrane (Spanner, 1954). Such processes of anomalous osmosis may create a difference in the activity of water on opposite sides of a membrane, but, as with other forms of diffusion, they can only bring about a continuous net transport so long as metabolic processes maintain the gradient of temperature or of electrical potential.

6. *Osmotic Pressure*

A solution in which the activity of the solvent has been depressed by the presence of solute cannot be in equilibrium with the pure solvent at the same temperature and pressure. If the solution and the solvent are brought into contact through a membrane permeable only to the solvent, osmosis occurs. The activity of the solvent in the solution can however be raised by increasing the hydrostatic pressure upon it, and if the excess pressure is just enough to compensate for the lowering of the solvent activity by the solute, a partial equilibrium (osmotic equilibrium) can be established. The criterion of exact compensation is the absence of any net movement of solvent across the membrane, i.e., that osmosis shall not occur. The excess pressure which just suffices to prevent osmosis defines the "osmotic pressure" of the solution. Osmotic pressure thus defined as that pressure needed to prevent osmosis is clearly *not* the cause of osmosis ; and indeed it is only manifested under the very special conditions of osmotic equilibrium, when no osmosis is occurring. Guggenheim (1933) pointed out that while osmosis is going on the pressure on the membrane may be trivial in magnitude and uncertain in direction, and further that the osmotic pressure of a solution bears no more relation to its actual pressure than does its freezing point to its actual temperature. The osmotic pressure, being the pressure required to prevent net diffusion of the solvent into a solution through an ideal semipermeable membrane, is a precise measure of the extent to which solutes depress the solvent activity, and as such it is related to other such measures as the lowering of the freezing point and of the vapor pressure of the solvent. Just as the freezing point can only be defined in the context of equilibrium between two phases, liquid and solid ; and the vapor pressure in the context of equilibrium between two phases, liquid and gaseous ; so also the osmotic pressure can only be defined in relation to the equilibrium between two phases, though in this case both are liquid.

This conception of osmotic pressure seems closer to the facts than the older analogy with gas pressure which persists in physiological literature (Chinard, 1954 ; Hildebrand, 1955 ; cf. Clark, 1948). It has the advantage of directing attention to the solvent, which undergoes net transport in osmosis, rather than to the solute, which does not. Moreover it leads, as an approximation valid for dilute solutions, to the familiar van't Hoff relation between osmotic pressure and solute concentration. The still incomplete thermodynamic treatment of the osmotic pressure of real solutions lies far beyond the scope of this chapter. Useful introductory accounts may be found in Alexander and Johnson (1949), Gutfreund (1950), and Moelwyn-Hughes (1957).

The van't Hoff formula $\Pi = RTc$ gives the osmotic pressure as proportional to the molar concentration of the solute. Taking R as 8.3147 joules per mole per degree and one atmosphere as 1.01325×10^2 joules per liter (Guggenheim, 1957, p. xxiii), the osmotic pressure of a solution containing one mole per liter at 37° C (310° K) should be

$$\frac{8.3147 \times 310}{1.01325 \times 10^2} = 25.4 \text{ atmospheres, approximately.}$$

Most body fluids, with concentrations of the order of 0.3 osmoles per liter, have osmotic pressures of the order of 7.6 atmospheres—over 7000 mm of mercury. Although measurements of osmotic pressure might therefore be expected theoretically to provide an extremely sensitive method for the determination of solvent activity in solutions, they are rarely employed except in the case of large molecular and colloidal solutes whose solutions have inevitably only minute molar concentrations, so that the other colligative properties are too small to measure and high sensitivity is valuable. The method is unsuited to the study of most body fluids because of the inconveniently high pressures and the enormous difficulty of producing truly semipermeable membranes for ions whose diameter is little greater than that of the solvent molecules.

The boundaries between a solution and ice or vapor in equilibrium with it provide more practicable semipermeable layers than any which can readily be devised for use between two liquid phases, and so it is customary to measure the depression of the freezing point, or less often the depression of the vapor pressure or the elevation of the boiling point, rather than the osmotic pressure, in order to discover the concentration of a solution in terms of the depression of the solvent activity. These methods are more convenient but less sensitive; the freezing point of water in an ideal molar solution is depressed 1.86°C, and the vapor pressure is depressed sufficiently to raise the boiling point at atmospheric pressure 0.52° C. Although the osmotic pressures of body fluids are hardly ever measured directly, the term " osmotic pressure " is commonly used as a colloquial expression for a total concentration arrived at cryoscopically, or sometimes for an osmolarity computed from the composition determined by analysis. Despite its lack of precision, this use of the term has some merit to denote the concentration of all solutes regardless of their chemical nature when it is desired to emphasize their influence upon exchanges of water.

It has recently been suggested that a difference in solvent activity may lead to something akin to bulk flow as well as to net transport by diffusion.† This belief appears to be based upon the observations that the rate of net transport through membranes under gradients of hydrostatic pressure (Mauro, 1957) or between liquids differing in osmotic pressure (Durbin *et al.*, 1956) may be far greater than the rates expected from measurements of the diffusion of isotopically labeled water through the same membranes. Kuhn (1951) in examining the extent to which osmotic and hydrostatic gradients might be considered equivalent, denied the possibility of bulk flow obeying Poiseuille's law through pores smaller than many molecular diameters. Bulk flow of solution is indeed inconceivable through an ideally semipermeable membrane, and such pores as there may be in cell membranes which maintain a functional impermeability to ions are also probably too small to permit bulk flow. Moreover it is not clear how a gradient of activity of the solvent could yield a force to move the solution bodily, although it might conceivably lead to a movement of the molecules of the hydration shell lining a pore, analogous to the surface migration of adsorbed molecules discussed by de Boer (1953).

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† See Vol. 1, Chapter 6.

The question arises whether the conditions of the tracer experiments and the measurements of hydrostatic or osmotic flow are sufficiently comparable to justify the conclusion drawn from the discrepancy between their results. Ussing (1952) regarded just such a discrepancy as a criterion of the existence of pores in a membrane. The essential difference between the two kinds of measurement seems to be that if THO or D_2O is genuinely acting as a tracer, there is no gradient of activity of *water*, and its transport ought to be slower than when differences in osmolar concentration or hydrostatic pressure create a gradient of activity. Harris (1956) pointed out that the molecular collisions around the opposite ends of the pores are similar in the tracer experiments, so that tracer molecules will tend to be jolted in and out of the pores at one end but will relatively rarely get right across the membrane. Collisions in experiments using osmotic or hydrostatic pressure gradients involve molecules differing in nature or energy, whence arises a greater tendency for the file of molecules occupying a pore to be transported across the membrane. Hodgkin and Keynes (1955) encountered a similar " queue effect " in the transport of potassium ions across the axonal membrane and illustrated it with a mechanical model.

7. *Ultrafiltration*

If a hydrostatic pressure greater than the osmotic pressure is applied to a solution confined by a semipermeable membrane, the activity of the solvent will be increased more by the applied pressure than depressed by the solutes, and this will cause a net flux of solvent through the membrane out of the solution, which will become more concentrated. This separation of a homogeneous liquid into its components is termed " ultrafiltration ", and is analogous with the separation of solid particles from a heterogeneous system by ordinary filtration. Pressures greater than seven atmospheres would be required to separate pure water from most body fluids, so that ultrafiltration in biological systems usually occurs across membranes which are permeable to all but a few solutes of high molecular weight. The effective osmotic pressure which has then to be exceeded to bring about ultrafiltration is the "colloid osmotic pressure," which in the case of the plasma is about 25mm Hg and is due to the plasma proteins. The ultrafiltrate resembles a protein-free plasma, so that this example of ultrafiltration is more closely analogous to everyday filtration through filter paper, but the analogy is not perfect because the composition of the fluid which crosses the membrane is somewhat altered. This might be expected in ultrafiltration through pores too small to permit bulk flow through the membrane to occur, for the rates at which the low-molecular components would pass through by diffusion would depend upon the effect of hydrostatic pressure upon their activities. Since the effect of pressure upon the chemical potential of each component depends upon its partial molar volume, all would not be expected to diffuse at the same rate.

8. *Membrane Equilibria*

When colloidal *ions* are confined to one side of a membrane, oppositely charged diffusible ions become more concentrated on this side and ions of like charge on the opposite side. The unequal distribution gives rise to a membrane potential which is simply related to the activity ratios of the diffusible ions at equilibrium and may be calculated from the Gibbs-Donnan distribution dealt with in all standard texts, e.g. Hitchcock (1945). Hitchcock's (1954) demonstration of a similar potential difference in the absence of a membrane, when diffusion is restricted only by the large size of the colloidal ions, shows that the mode of restraint is relatively unimportant. Similar distributions may be established whenever an ionic species is immobilized, by whatever means, and Danielli's application of this principle to the distribution of ions between a surface and an adjacent bulk phase has been mentioned already.

Although membrane distributions are sometimes reminiscent of the differences in composition between glandular secretions and the blood plasma, continuous secretion requires an output of work which no system in equilibrium can supply, and membrane equilibria are not sufficient to explain secretion. They may however influence the composition of ultrafiltrates, which, if formed slowly enough, should approach the composition expected at equilibrium. Membrane equilibria also affect the distribution of water between compartments differing in protein content because the excess concentration of diffusible ions on the same side of a membrane as the colloidal ions depresses the activity of the solvent, and the measured "colloid osmotic pressure" is greater than corresponds to the molecular concentration of the colloid. This effect is greatest with low concentrations of salts (Hitchcock, 1945) ; it accounts for about one-third of the "colloid osmotic pressure" of the plasma.

C. Active Transport

Since much detailed information about active transport is contained in the preceding Chapter, "Ion Transport," no more is required here than the briefest general definition. Rosenberg (1954), Wilbrandt (1954), and Ussing (1954), in contributions to a *Symposium of the Society for Experimental Biology*, seemed to be in substantial agreement that the simplest definition of active transport is transport across cell membranes, brought about by means of energy from metabolic sources rather than by passive processes such as diffusion under the influence of gradients of chemical potential. Active transport is characteristically dependent upon continuing metabolism and vulnerable to metabolic poisons ; it often shows structural specificity and competitive inhibition by chemically related substances ; its rate is not a linear function of concentration and tends to have a high temperature coefficient. But although these are all suggestive properties, they are not sufficient to establish that transport is active rather than passive. To establish beyond cavil that a transport process produces a movement away from chemical equilibrium or maintains a steady state removed from equilibrium, it needs to be shown that transport takes place in the absence of a gradient of activity which could account for it as a spontaneous process, or better that it takes place in spite of a gradient which would cause spontaneous movement in the opposite direction. [The theoretical possibility that an active process might assist a spontaneous change must be admitted, but it would be difficult to demonstrate (cf. Ussing, 1949)].

For nonelectrolytes this last requirement is satisfied if a substance is transported from a region of low to a region of higher concentration. The situation for ions is complicated by the presence of charges on the membranes, for an ion might be accumulated passively by a cell because of an opposite charge within the membrane. The test of active transport of an ion is therefore movement to a region of higher electrochemical potential ; this takes account of the effects of charge as well as of concentration. Ussing (1952, 1954) has developed the employment of isotopically determined flux ratios as an alternative criterion for ions. To establish active transport of water it would be necessary to demonstrate continuous movement into a region of the same or a greater activity of *water*, that is, net movement without or against an osmotic gradient ; or else the maintenance of a difference in osmotic pressure across a membrane permeable to water.

It will be seen that, in contrast to passive, "down-hill" transport by diffusion, active transport is net transport "up-hill" against gradients of activity or electrochemical potential. It is fundamentally important as the means of maintaining as steady states those differences in concentration which enable most of the exchanges of matter in the body to be effected by diffusion. If these concentration gradients are the mainspring of most transport processes, active transport keeps them wound. Since cell membranes are permeable to the common mineral ions of the body, the profound differences in composition between intracellular and extracellular fluids must depend upon active transport of these ions. Details of the active transport of individual mineral ions will be found in the appropriate chapters of this book. The question whether water, as well as solutes, is subject to active transport is not dealt with elsewhere and will be briefly considered in Section VII of the present chapter.

V. Exchanges between Organism and Environment

Mammals meet their environment and exchange matter and energy with it at the body's surfaces. The general body surface permits mainly exchanges of heat, which is conducted across the skin by a process analogous to diffusion. Matter is exchanged in the lungs, the alimentary tract, and the kidney, where the extracellular fluid faces the environment across specialized frontier membranes.

A. Skin†

The outer layers of the skin form an almost waterproof coat restricting fluid loss. But since the surface is moist, water, without solute, is lost spontaneously as vapor and is replaced from the deeper layers by diffusion. The rate of the primary process of evaporation depends upon the temperature of the skin, which fixes the vapor pressure, and upon the saturation of the surrounding air. This loss by evaporation is inevitable, and unrelated to the body's need for water; it ordinarily amounts to one-third to one-half of a liter per day: and the body loses the 200–300 cal of heat absorbed in its vaporization.

The skin is pierced by the ducts of sweat glands, through which water is lost, together with solutes, at a rate controlled by the nervous system so as to regulate the temperature of the body; this too is an inevitable loss which bears little relation to the body's need for water and may exceed 10 liters daily in men exposed to hot climates. The principal solute is sodium chloride, but the molecular concentration of the sweat is less than half that of the plasma, so that it must be produced at the expense of metabolic energy in the sweat glands. The customary distinction between sweat and "insensible" perspiration is not altogether satisfactory, for sweat may be "insensible" if it evaporates as fast as it is formed. Losses through the skin might be classified as transpiration i.e. net transfer by diffusion of water unaccompanied by solute, to replace water which has been lost as vapor, and perspiration, i.e. the loss from the sweat glands of a specific liquid secretion which may or may not subsequently evaporate.

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† Cf. Vol. 1, Chapter 10.

B. Respiratory Tract

Unless the inspired air is saturated at body temperature, it acquires water from the upper respiratory tract and presumably becomes completely saturated in the pulmonary alveoli, which allow gaseous exchange to occur and present an extensive moist surface from which water may evaporate and be replaced from the blood by diffusion. The expired air, being a mixture of alveolar air with air from the respiratory dead space, may not be completely saturated at body temperature (two-thirds saturated according to Brebbia *et al.*, 1957) but will usually contain more water vapor than the inspired air, so that water is continually lost from the respiratory tract. This loss is ordinarily of the same order as the loss by transpiration through the skin and is similarly an inevitable loss, unrelated to the body's need for water and accompanied by loss of the latent heat of vaporization. The losses of water and heat may become critically important during high altitude mountaineering when the ventilation rate has to be increased enormously to supply oxygen.

Although water is lost from the lungs, Meschia and Barron (1956) found that dog arterial plasma had a *lower* molecular concentration than venous plasma, mainly on account of the elimination of carbon dioxide. The 15–30 osmoles of carbon dioxide produced each day by a man are excreted by the lungs, while less than a liter of water is lost by evaporation, so that the lungs eliminate a far larger daily osmotic load than the kidneys, and with a remarkable economy of water.

There is normally no important net exchange of water in liquid form across the alveolar membrane. Pressure in the capillaries is low because they communicate with great vessels exposed to a subatmospheric pressure within the thorax, whereas the alveolar capillaries themselves are exposed to an external pressure very close to atmospheric. Consequently the hydrostatic pressure difference across the capillary membrane is well below the colloid osmotic pressure of the plasma, conditions are unfavorable for ultrafiltration, and the alveoli are kept clear of free fluid. Chinard and Enns (1954) have presented evidence that the alveolar membrane is far more permeable to water than to ions, and osmotic exchanges may occur so rapidly when liquid reaches the alveoli that the immediate cause of death in drowning may be inundation of the blood stream with fresh water or sudden hemoconcentration from the osmotic effect of sea water (Crossfill, 1956 ; Donald, 1955).

C. Alimentary Tract†

The alimentary tract presents a large area of epithelium specialized for the absorption of water and substances in solution. The associated digestive glands pour into it about 8 liters daily of mixed secretions, most of which are subsequently reabsorbed. The absorption of the various mineral elements is dealt with in later chapters. Water is believed to be absorbed passively by diffusion because of osmotic gradients between ingested fluid and the blood or created by the active absorption of solutes, as was shown particularly clearly in the rat by Curran and Solomon (1957). Apart from this there does not appear to be any important physiological control of the absorption of water at the intestinal level ; what is ingested and passed on by the stomach is absorbed, but the nervous system through habit and thirst controls the volume ingested.

D. Kidney‡

Each of the million or so functional units of a mammalian kidney includes a tuft of glomerular capillaries from which a relatively protein-free fluid emerges, to be converted into urine by the reabsorption of most of the water and mineral solutes as it moves along the renal tubule. The fluid first formed is universally called the glomerular filtrate. It is certainly not a filtrate ; it has approximately the composition of an ultrafiltrate, although there is some doubt whether it is formed by ultrafiltration or by diffusion (Chinard, 1952). In either case, the energy for its formation is the potential energy of glomerular capillary blood pressure and is supplied by the heart, not by the kidney. Flow along the tubule is bulk flow under the influence of the residual pressure gradient. Tubular reabsorption at least of some solutes depends upon active transport by the epithelial cells and is considered in later chapters. Although the kidney is primarily an excretory organ, a great part of its active secretion is directed from lumen to blood, like that of the alimentary tract. The urine is made up for the most part of unabsorbed residues from the glomerular fluid, although some of its constituents, including potassium, may be actively secreted into the tubular fluid by the epithelial cells.

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† See also Vol. 1, Chapter 8.

‡ See also Vol. I, Chapter 9.

From the restricted viewpoint of body fluid dynamics, the kidneys are the organs which control losses of water and of solutes in solution from the extracellular fluid to the environment and adjust both the concentration and the volumes of the principal fluid compartments of the body. An antidiuretic hormone released by the neurohypophysis when the osmolarity of the plasma tends to rise increases the tubular reabsorption of water so that less escapes into the urine†. Increasing osmolarity of the plasma also evokes thirst, leading to the ingestion of water which is retained by the kidney until the osmolarity of the plasma begins to fall again (Verney, 1946, 1947, 1957). Because, as will become clear in Section VII, the osmolarity of the extracellular fluids is the most important single factor determining exchanges between the intracellular and extracellular compartments, this mechanism, by acting upon the extracellular fluid to fix its molecular concentration, also regulates the volume of water in the cells. Moreover since 90% of the molecular concentration of the extracellular fluid is due to sodium salts, the kidneys, granted a sufficient dietary intake of sodium, can further regulate the volume of the extracellular compartment by controlling the excretion of sodium. This they appear to do in response to alterations in the volume of blood or of total extracellular fluid by mechanisms which are discussed in Chapter 12. The physiology of the kidney is dealt with fairly briefly by Wolf (1950), Robinson (1954), and Smith (1956); see also Vol. I, Chapter 9 for further references.

VI. Exchanges between Extracellular Phases

A. Plasma and Interstitial Fluids

Only the capillaries have thin enough walls to permit rapid exchanges between plasma and extravascular fluids. All of the water and low-molecular solutes of the plasma exchange by diffusion through the capillary wall at least about once each minute, water diffusing approximately twice as fast as the solutes (references in Yoffey and Courtice, 1956). Pappenheimer *et al.* (1951) suggested that the rates might be as much as one hundred times greater. In contrast with the rapid, and nearly equal, fluxes by diffusion in opposite directions, net exchanges affect only a few percent of the plasma constituents each minute.

There are two opposed views on the mechanism of exchange. The capillary membrane may be a sieve which allows low-molecular substances to exchange through pores in an intercellular cement, while the cells themselves show a typical functional impermeability to inorganic ions (Chambers and Zweifach, 1947). Equilibrium across the capillary wall then depends upon balancing hydrostatic against colloid osmotic pressures. According to the "Starling concept," an ultrafiltrate is formed at the arterial ends of the capillaries, where excess hydrostatic pressure exceeds the difference in colloid osmotic pressure between the plasma and the fluid outside, and re-enters by osmosis at the venous ends where the balance of pressures is reversed. This hypothesis is supported by a great deal of evidence concisely reviewed by Yoffey and Courtice (1956), and Starling's (1898) account of the mechanism is still well worth reading. In the alternative hypothesis, all constituents of the plasma exchange across the whole surface of the capillary wall by diffusion according to their activities and their coefficients of restricted diffusion in the substance of the wall (Chinard, 1952; Chinard *et al.*, 1955). Since the activities are influenced by hydrostatic pressure, both mechanisms can lead to somewhat similar results. It is more than likely that exchanges occur by both processes, and Pappenheimer (1953) has discussed the relations between them.

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† See also Vol. 1, Chapters 1 and 12.

Most of the interstitial fluid outside the capillaries appears to be enmeshed in a connective tissue gel (Robb-Smith, 1954), and little is free to flow, although the gel is probably dilute enough to restrict diffusion only slightly. Moreover, capillaries little, if any, greater in diameter than erythrocytes can hardly be regarded as tubes through which fluid flows steadily with a gradually diminishing pressure. A steady fall in pressure is more likely in thoroughfare channels than in true capillaries, where flow seems to be intermittent, and not even constant in direction. Temporary stasis would favor exchange by diffusion rather than by ultrafiltration, especially as the hydrostatic pressure in the true capillaries is probably low. Chambers and Zweifach (1947) suggested that the true capillaries might reabsorb ultrafiltrate formed in the thoroughfare channels where hydrostatic pressure is higher. Sufficiently prolonged stasis would allow all species, including proteins, to approach equilibrium by diffusion. Burton (1954) suggested, as an explanation of benign (orthostatic) albuminuria, that stasis would allow a greater proportion of elongated molecules to enter pores because they would be orientated at random instead of predominantly in the streamlines (the mechanism of such orientation was investigated by Robinson, 1939).

Protein cannot be expected to re-enter capillaries either by diffusion or by filtration; and its accumulation outside would soon equalize the colloid osmotic pressure on either side of the capillary wall, so that water and low-molecular solutes would also cease to return. Hence a normal exchange of fluid across the capillary membrane depends upon the continuous removal of protein which has left the capillaries. The terminal lymphatics appear freely permeable to protein, which is drained from the interstitial spaces and returned through valved channels to the great lymph ducts which empty into the central veins. This is transport by bulk flow, which depends upon residual pressure derived ultimately from the heart, compression of lymphatic vessels by the contraction of muscles and the pulsation of neighboring arteries, and a low intrathoracic pressure maintained by the diaphragm and the elasticity of the lungs and thoracic cage.

If the regional lymph reflects the composition of the extravascular fluid, then this varies a great deal in protein content from one region to another. The concentration of protein in the interstitial fluid must depend upon the rate at which the lymphatics remove protein as well as upon the rate at which it leaves the capillaries. A small difference between the concentrations in lymph and plasma does not necessarily imply a high permeability of the local capillaries to proteins. Permeability may be high in the liver, although the formation of plasma proteins may contribute to the high protein content of hepatic lymph ; the small difference in colloid osmotic pressure between plasma and extravascular fluid is also related to the low hydrostatic pressure in the hepatic capillaries, perfused through the portal vein by blood which has already traversed another capillary bed. When, as in the liver, lymph and plasma have similar concentrations of protein, caution is required in applying customary " Donnan ratios " to calculate the concentrations of diffusible ions in the interstitial fluid from those in the plasma (Harrison, 1953). The pulmonary lymph also has a high protein content, although the permeability of the capillaries to substances other than water is probably unusually low (Chinard and Enns, 1954). The small difference in protein concentration between lymph and plasma is probably related to the low pressure in the pulmonary capillaries and the slow flow of lymph compared with the rapid circulation of blood through the lungs.

Exchanges across the capillary membrane do not appear to involve active transport.

B. Plasma and Transcellular Fluids

Of transcellular fluids which are truly inside the body, most useful information is available about the ocular and cerebrospinal fluids. These differ from the common interstitial fluid in being separated from the plasma not only through the capillary membrane but also through a layer of specialized cells in the ciliary body or choroid plexus. They are moreover free to flow more or less unhindered by a gelatinous matrix and have a definite circulation with channels for drainage remote from the site of their formation. Even the capillaries where they might reach equilibrium with the blood by diffusion seem to possess a peculiarly restricted permeability. Krogh (1946) drew attention to the long times taken by anions like bromide, iodide, thiocyanate, and chloride in the plasma to attain equilibrium with the same ions in the cerebrospinal fluid and to the even longer time taken by sodium.

Both fluids somewhat resemble an ultrafiltrate of plasma in that they contain about 200 times less protein per unit weight of water but diverge far less in respect to diffusible constituents. They were formerly considered to be ultrafiltrates because the concentrations of sodium and chloride in them agreed with those predicted for a membrane equilbrium by the use of theoretical Donnan ratios. Davson (1954, 1956) pointed out, however, that they did not so closely resemble actual dialyzates and that their compositions were altered by dialysis against plasma with which they cannot therefore have been in equilibrium. Thus aqueous humor from dogs and cats lost sodium and chloride when dialyzed against plasma. The cerebrospinal fluid of the rabbit contains about 8% more sodium, 16% more chloride and 40% less potassium than an ultrafiltrate ; and although aqueous humor shows far smaller differences in the concentrations of these ions, it contains about 25% more bicarbonate per unit mass of water than the plasma besides about 18 times as much ascorbic acid. Both fluids contain significantly lower concentrations than the plasma water of glucose and even of the highly diffusible urea. Davson (1956), in a detailed discussion of their formation, pointed out that neither fluid could be produced by ultrafiltration through a selectively permeable membrane with the pressure available in the capillaries. Although an additional force moving water and solutes might be derived from active transport of sodium salts, which would lower the activity of water and cause its transfer by osmosis, this could hardly account for the movement of other solutes with the water and could by no means account for their being concentrated like ascorbic acid in the aqueous humor.

It seems clear that the ocular and cerebrospinal fluids are specific secretions produced by the expenditure of energy released within the secreting cells. Exchange between the plasma and these transcellular fluids therefore differs fundamentally from the exchange between the plasma and interstitial fluid in being dependent upon active transport. Further details will be found in the chapters devoted to individual ions. Davson and Purvis (1954) found that the molecular concentration of rabbit cerebrospinal fluid, determined cryoscopically, was 3–4% greater than that of aqueous humor or of an ultrafiltrate of plasma, but this need not imply active transport of water. In view of the low permeability to sodium of capillary membranes through which cerebrospinal fluid might equilibrate with the plasma, it could be accounted for by active transport of sodium ; and the entry of water by osmosis may be a factor in maintaining the normal pressure and circulation of the cerebrospinal fluid.

VII. Exchanges between Cells and Extracellular Fluids

A. Introduction

Exchanges between intracellular and extracellular fluids involve both solvent and solutes and might be effected by active as well as by passive transport. The simplest view is that active transport of inorganic ions enables the cell membranes to behave as more or less semipermeable and that alterations of cellular water balance are effected by passive transfers to eliminate osmotic gradients.

Passive exchange of water between cells and extracellular fluid can take place only by diffusion across the cell membrane, and net exchanges must depend upon differences in the activity of water inside and outside the cells. The activity of water outside the cells depends chiefly upon the concentration of sodium and accompanying anions and is held remarkably constant by the combined operation of thirst, which guards against a decrease in the activity of water, and of the kidneys, controlled by the hypothalamic-hypophyseal antidiuretic mechanism (Verney, 1957) which guard against an increase in the activity of water. The extracellular fluid thus provides a bath of constant osmotic pressure which mediates the kidneys' contribution to stabilizing the volume of the cells.

Since the volume of a cell in osmotic equilibrium with a constant extracellular osmotic pressure must be proportional to the amount of osmotically active material inside it, metabolic activity is a most important internal factor controlling passive exchanges of water between cells and their surroundings. This is so because the ion pumps responsible for extrusion of sodium and the accumulation of potassium depend upon energy from metabolic reactions and also because organic compounds may be produced or destroyed as they undergo metabolic transformation. Hence metabolic events profoundly influence the activity of water within the cells.

B. Osmotic Behavior of Cells

If all exchanges of water were passive and all cell membranes ideally semipermeable, cells should behave as osmometers. An ideal osmometer, which may be defined as a quantity of solution enclosed within a semipermeable membrane and free to change its volume, swells in solutions more dilute than its contents and shrinks in stronger ones, but undergoes no alteration of volume in any isosmotic solution. Cells cannot be expected to behave as ideal osmometers, for their membranes are permeable in varying degree to most of the solutes encountered in the body. Accordingly, the net exchange of water between a cell and an external solution cannot always be predicted from the osmotic pressure of that solution. Solutions in which cells neither shrink nor swell are said, by definition, to be isotonic ; cells swell in hypotonic and shrink in hypertonic solutions just as osmometers swell in hypoosmotic and shrink in hyperosmotic solutions. Solutions isotonic for the same cell need not have the same osmotic pressure with reference to an ideal semipermeable membrane. Thus erythrocytes may swell and burst in a solution of urea or glycerol which has the same osmotic pressure as a salt solution with which they would be in equilibrium ; and a strongly hyperosmotic solution made by adding urea or glycerol to isotonic saline may provoke an osmotic transient but appear isotonic if time is allowed for equilibration (Davson and Danielli, 1943). The erythrocytes first shrink in such solutions as the penetrating solute reduces the activity of water outside them and then swell again as it enters and reduces the activity of water inside.

There is evidence that nucleated cells as well as erythrocytes behave a good deal like osmometers. For example, Winkler *et al.* (1944) raised the concentration of extracellular electrolytes and found that water moved out of cells, while Darrow and Yannett (1935) found that water moved into cells when extracellular electrolyte was removed from experimental animals. Evidence that the same is true in man has been reviewed by Wynn (1957); cf. Edelman *et al.* (1958).

Although cells *in vivo* may respond like osmometers to alterations in external concentration, the shifts of water are often smaller than would be expected (Mellors *et al.*, 1942 ; McDowell *et al.*, 1955). The discrepancy may be partly attributed to permeability of the membranes, but metabolic processes also play an important part in restricting alterations in the volume of cells. This effect has been demonstrated in the whole animal (Welt *et al.*, 1950 ; Hamburger and Mathé, 1951), but it is most clearly revealed by the behavior of isolated tissues *in vitro*. When tissues are studied under conditions which preclude normal respiration, far stronger solutions are required to prevent swelling than the body fluids with which the cells were in equilibrium during life (Opie, 1955). Swelling may be prevented, and solutions of the same concentration as normal extracellular fluids may also function as isotonic *in vitro* if respiration is allowed to continue normally, though a large reversible uptake of water follows inhibition by chilling, lack of oxygen, or metabolic poisons such as cyanide or 2,4-dinitrophenol (Robinson, 1950a, b, 1952). The most widely accepted explanation of this swelling is that when active transport of ions ceases, potassium leaves the cells and sodium enters. Usually more sodium enters than potassium leaves (Mudge, 1951), and chloride from the medium enters along with it, so that the amount of osmotically active solute in the cells is increased and water must enter to maintain osmotic equilibrium. This appears to have been best established for rat diaphragm by Rixon and Stevenson (1956, 1957). Leaf (1956) sought to explain the swelling of kidney slices when metabolism was suppressed by uptake of isotonic sodium chloride from the medium ; but an essentially similar uptake of water may occur from media which have choline sulfate as their principal solute and contain no sodium chloride to enter the cells. Changes akin to those known to occur during autolysis might also increase the amount of osmotically active material in the cells and contribute to this swelling, but less is known about this possibility (Robinson, 1956).

C. Osmotic Equilibrium between Cells and Surroundings

Living cells behave sufficiently like osmometers to make it certain that their membranes are permeable to water. This was confirmed by observations like those of Hevesy and Jacobsen (1940) and of Flexner *et al.* (1942), which showed that deuterium oxide may become distributed throughout the entire body water of experimental animals in a few minutes. Such free permeability of the cell membranes to water makes it unlikely that cells and their surroundings are ever far from being in osmotic equilibrium. It is indeed generally taught that " despite the extreme differences in the composition of its fluid compartments, a uniform osmotic pressure prevails throughout the fluids of the body " (Peters, 1935, 1944) ; but in view of the influence of physiological activity upon metabolism, and so upon the activity of water in the cells, Manery's (1954) version, " Osmotic equilibrium is probably never attained but always approached," may be preferable.

The large gradients of concentration of sodium and potassium maintained by living cells across membranes which are permeable to both ions should, however, be a sufficient warning that it does not *necessarily* follow from the permeability of their membranes to water that cells are in osmotic equilibrium with their surroundings. McDowell *et al.* (1955) remarked that " it may be true or not that osmotic pressure within cells exceeds that outside of cells ; but neither case is necessarily incompatible with the findings that cells act volumetrically as if they were osmometers when osmotic stresses are applied " (cf. Wolf, 1956).

No unexceptionable measurement has yet been made of the activity of water in *any* living mammalian cell to prove directly the statement that a uniform osmotic pressure prevails throughout the extracellular fluids and the intracellular fluids of *all* cells. The most direct measurements have been made by cryoscopy on tissue disintegrated by grinding. There is a risk of supercooling in mixtures containing colloids ; Kuhn and Majer (1955) showed that the freezing point of pure water enmeshed in gels of polyacrylic acid or polyvinyl alcohol might be depressed as much as 1–2° C. The method is also liable to overestimate the osmolarity of cell fluids during life because of autolysis during the preparation of the tissue. Conway *et al.* (1955) showed that autolytic changes, principally breakdown of phosphoric esters, went on with hitherto unsuspected rapidity in ground rat tissues even at 0° C. This may partly explain why earlier workers (see Robinson, 1953) had concluded that the osmolarity of the cell fluids of normal parenchymatous organs was 50–100 % greater than that of the plasma. Conway and McCormack (1953) seem to have been the first to be able to show cryoscopically that mammalian tissues and extracellular fluids were isosmotic, and Conway and Geoghegan (1955) found that incubated kidney slices had the same freezing point as the medium if allowance was made for autolysis. These kidney slices had been kept on ice for an hour, and they had the same total concentration of Na plus K as the medium. It therefore remains possible that slices incubated under better conditions by Aebi (1953) and which maintained a concentration of Na plus K 90 % greater than that of the medium had also a higher osmolarity. Although the doubt raised by Aebi's experiments with tissue slices still remains to be resolved, the most recent experimental evidence has increasingly favored the view that intracellular and extracellular fluids have the same osmotic pressure, at least in tissues shortly after they have been removed from intact animals. Buckley *et al.* (1958) have answered criticisms by Brodsky *et al.* (1956) of the results of Conway and his co-workers; and measurements by Maffly and Leaf (1958, 1959) and by Appelboom *et al.* (1958) using new methods have substantially confirmed Conway's results. The whole question of the osmotic equilibrium of cells and their surroundings has been discussed in greater detail in a recent review (Robinson, 1960).

No certainty about the equality of intracellular and extracellular osmotic pressures can yet be reached by way of chemical analysis. The difficulty, already mentioned, of precisely calculating the osmotic pressure of the cell fluids from a detailed knowledge of their composition has been tremendously increased by the general acceptance of a suggestion by Elkinton *et al.* (1944) that some discrepancies between cellular exchanges of water and cations were too great to be ascribed to experimental error, and must imply that a variable fraction of intracellular "base" was bound in some osmotically inactive form. Since this inactivated base has not been characterized chemically, its quantity can only be deduced by assuming osmotic equilibrium, with the consequence that analytical results cannot be used to prove the assumption.

Like the idiogenic increase in intracellular osmotic pressure postulated by McDowell *et al.* (1955), the reactivation and inactivation of cell constituents could provide a mechanism of osmotic buffering, protecting the cells from changes in volume imposed by fluctuations in external osmotic pressure. The same discrepancies which led these hypotheses to be put forward could also be explained if the cells possessed a measure of osmotic independence through being able to transport water actively, as was suggested some years ago in attempting to explain the reversible swelling of kidney slices when their respiration was impaired (Robinson, 1950a). The behavior of isolated tissue slices in media lacking the usual extracellular ions (Robinson, 1956) and the observation that oxygen supplied in liquid paraffin led to the removal of water from swollen cells in the absence of the usual external aqueous phase to provide a gradient of activity across the cell membrane (Robinson, 1957) suggest that the mechanisms whereby respiration normally restricts the net entry of water into cells involve something more than the prevention of autolytic changes and the maintenance of a normal distribution of ions.

Active transport of water has not been proved to occur because the activity of water in a living mammalian cell has not yet been accurately determined. For precisely the same reason it has also not been proved not to occur. Active transport of water across mammalian cell membranes may be *a priori* improbable, but it is not conclusively disproved, whereas the alternative assumption of osmotic uniformity, which is generally accepted because of the simple explanation it affords of the gross behavior of cells, is inherently probable, but not rigorously proven.

Most net movements of water between cells and extracellular fluids are the result of passive transport. But there appear to be some obvious exceptions to the statement that all cells are in osmotic equilibrium. Protozoa living in fresh water keep their contents more concentrated than their surroundings. Cells which live between solutions differing in concentration, like those lining the frontiers between aquatic metazoa and their habitat or lining the cavities of some mammalian glands, can hardly be in equilibrium with both simultaneously. Though most mammalian cells, protected as they are by the controlled osmotic pressure of the extracellular fluids, may not share the ability of some more primitive cells to control their water content actively, it seems hard to account for the production of strongly anisotonic secretions without active transport of water.

This section can be summarized by stating that net movements of water between the intracellular and extracellular compartments of the mammalian body may result from four main causes.

1. Net movements of ions. Some of these are active (cf. Vol. I, Chapter 6) and are probably the principal processes responsible for shifts of water. Whether active or passive they tend to cause net movements of water in the same direction by osmosis.

2. Changes in the amount of organic metabolites in the cells. Little is known about these changes, but autolytic changes may serve as models. These changes tend to produce permanent shifts or osmotic transients according to their own persistence and the permeability of the membranes to the substances concerned.

When net exchanges resulting from the above causes are linked to metabolism, the transport of water may appear to be active ; but it is strictly a passive osmotic transfer secondary to changes in the activity of water produced by solutes.

3. Alterations in extracellular osmolarity. These also provoke passive osmotic transfers of water.

4. Active transport of water as such, by some process which can affect its activity directly, remains an additional possibility in some mammalian cells.

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CHAPTER 8

Intestinal Absorption and Excretion

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I. Introduction

If one plans to study the physiology of the intestinal tract, one must be prepared for a most elusive organ. The investigator who is trying to decide how things happen is confronted first with difficulties in deciding what happens. A glance at any series of repeated observations in gastrointestinal physiology commonly reveals a wide range of measurements for the same experiment. For example, the amount of chloride entering the dog ileum through the mucosa is apparently independent of the chloride concentration in the lumen, amounting to

26 μeq for each milliliter of water that enters, but the range about this mean is 0–83 μeq (Visscher *et al.*, 1944a). It thus becomes necessary for certain studies to select animals with relatively stable control values, and investigators have been forced to such qualifying statements as: " Out of a larger number studied, two animals showing the closest constancy of absorption rates under controlled conditions in repeated observations were selected for the main studies " (Dennis and Visscher, 1940).

Control studies with isolated loops of intestine have shown not only variations in unidirectional fluxes of sodium, chloride, and water into and out of the lumen, but the net flux may result in either an increase or a decrease of the amount of electrolyte in the lumen (Visscher *et al.*, 1944a, b; Berger *et al.*, 1959a). Hour-to-hour variability of unidirectional sodium flux across the intestinal mucosa of the dog has been shown to be of the same order of magnitude as the day-to-day or year-to-year variability of the same isolated loop observed over a four-year period (Berger *et al.*, 1959a). This inherent variability, the cause of which remains obscure, is the greatest obstacle to ascertaining the effect of drugs or procedures.

An evaluation of the results of investigations of a single question by somewhat different techniques leads to further uncertainties. The question of the effect of intralumenal hydrostatic pressure on the transfer of water would seem to be an uncomplicated one to answer and has been investigated thoroughly. Yet a study of the measurements of Wells (1931), Blickenstaff *et al.* (1952), Fisher (1955), Smyth and Taylor (1957), and of the discussion of Durbin *et al.* (1958) leaves one without knowing with certainty whether an increase in hydrostatic pressure does or does not increase water absorption.

The reports dealing with the intestine have been plentiful, but the dearth of clear-cut results has made it difficult to decide whether one or the other study should be discussed in this chapter, without becoming involved in a discussion of the (largely unknown) reasons for the discrepancies. Rather than merely reviewing the literature, the selection was directed toward a presentation of a cohesive story, based on many diverse, but not all, observations, minimizing the unsettled issues, and emphasizing the known.

II. Definition of Terms

The terms " unidirectional flux," " influx," and " outflux " deserve definition. " Unidirectional flux " refers to the one-way passage of electrolyte (or water) from one side of the intestinal mucosa to the other, which occurs concurrently with a one-way passage of the same ion in the opposite direction.† Inherent in this definition is the condition that these two fluxes occur within a limited area of mucosa which is histologically similar throughout.

The terms " influx " and " outflux " lead to considerable confusion, e.g., whether influx is into the intestinal lumen or into the animal (Berger *et al.*, 1959a, Tables 7 and 8). In general, with regard to the intestinal tract, influx has been flow into the lumen, while, with regard to the frog skin, influx has been flow into the animal. This chapter will follow convention and will use " influx " as " flow into," and " outflux " as " flow out of the intestinal lumen," and will specifically state the direction of flux, i.e., into or out of the lumen, unless it is otherwise obvious. Precise definition of the direction of flow is preferred to the terms influx and outflux ; e.g., " gut to blood " or " blood to gut " in reference to the dog intestine (Visscher *et al.*, 1944a, b), " nutrient to secretory " or " secretory to nutrient " in reference to the isolated gastric mucosa of the frog (Hogben, 1955). The terms " flux " or " flow " will be used only in the sense of " unidirectional " fluxes or flows.

Absorption for the purposes of this chapter is a net decrease in the amount of electrolyte or water in the lumen, resulting from a flux out of the lumen that is greater than the flux into the lumen. Similarly, secretion is a net increase in the amount of electrolyte in the lumen resulting from a greater flux into than out of the lumen. In the case of absorption, flux into the lumen may be zero ; likewise, in the case of secretion, flux out of the lumen may be zero.‡ Absorption and secretion will be used where the unidirectional fluxes are unknown (for example, secretion of potassium by the distal portion of the nephron).

Excretion refers to the final excretory product, i.e., the stool contents.

III. Methodology

Research concerned with the intestinal tract over the last two or three decades has been considered to have " retreated into a meditative phase " (Bucher *et al.*, 1950), and has been described as " stagnant " (Öbrink, 1958). In the light of this " meditative " and " stagnant " state of affairs, some emphasis will be placed on the methods of study. While methods may not be the means of lifting the research out of this alleged state, they are among the nutrients which supply the root tips of advancing knowledge. Furthermore, extending the section on methods provides an opportunity for including observations which do not naturally fall into other sections. The classification of the methods as presented here is arbitrary, for the procedures may well overlap different categories.

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† " Unidirectional flux " has been the usual term to describe the one-way passage of electrolyte across the membrane of the nerve fiber ; it is preferred to the term " bidirectional " which may connote a single source dividing into two directions.

‡ A zero flux probably exists only in terms of the precision of the measurement.

A. Balance Study

The simplest method of study of the intestinal tract is the " material balance " or " physiological disposition " study, where the substance of interest is fed by mouth and it or its by-product is looked for in the stool, blood, or urine. Secretion into the intestinal lumen may be demonstrated by administering the material or an isotopic tracer parenterally and by looking for its appearance in the stool.

Cheng and Stanley (1954) have used the chemically inert and nonabsorbable chromic sesquioxide as an indicator to avoid continuous stool collections in man. Once the subject reaches equilibrium, having been on a constant oral intake of chromic sesquioxide and of the material under study, the analysis for chromic sesquioxide and the material in a single stool specimen will allow calculation of the daily fecal excretion of the material. This method agrees well with the classical balance study for daily excretion of cholesterol and nitrogen (Stanley and Cheng, 1956). Liebowitz *et al.* (1957) used Zr^{95}-Nb^{95} as a volume indicator to measure residual fluid in the stomach, but the addition of a chelating agent was necessary to prevent adsorption of the Zr^{95}-Nb^{95} to the stomach wall. Presumably, Zr^{95}-Nb^{95} can substitute for chromic sesquioxide, if the chelating agent does not interfere with the planned investigations.

Studies which include a nonabsorbable indicator in a constant proportion in the diet have been used to decide whether there is net absorption or net secretion of electrolyte at a given level of the gastrointestinal tract. Rats which had been maintained on a constant diet containing 1% titanium dioxide as an indicator were sacrificed at the end of seven days, and the contents of various sections of the gastrointestinal tract were analyzed for calcium and titanium dioxide (Fournier, 1950b). The ratio of calcium to titanium in the diet was compared with that found in the intestinal lumen ; a dietary ratio exceeding the ratio found in the intestine indicated absorption, and the converse, secretion. The procedure assumes that the transit times for calcium and titanium dioxide are the same, but the fact that for varying intakes of titanium dioxide relative changes in the calcium/titanium oxide ratio remain the same at various levels of the gastrointestinal tract does not prove Fournier's assumption (1950a). Fournier (1950b) concluded by this procedure that a net secretion of calcium occurred in the first half of the small intestine. Bergeim (1926), in similar studies in rats with iron oxide as the indicator, concluded that a net absorption of calcium occurred in the second and third quarters of the small intestine.

B. Intubation

Intestinal intubation is one means of obtaining samples of the intestinal contents *in vivo.* In 1934 Miller and Abbott described a double lumen rubber tubing with an outside diameter of 6 mm, which can be passed into the intestinal tract of the human. Fluids can be instilled and samples removed through one lumen, while the other lumen leads to a terminal inflatable rubber bulb which is propelled along the intestinal tract by peristalsis. A number of modifications are available, e.g., single or triple lumen, mercury-weighted end, etc. (Abbott and Miller, 1936; Nicholson and Chornock, 1942; Cantor, 1949). More recently, Blankenhorn *et al.* (1955) used polyvinyl tubing (outside diameter, 2.1 mm) which has remained *in situ* in the human as long as 26 days without untoward effects. Borgström *et al.* (1957) used such polyvinyl tubing to administer polyethylene glycol (which is nonabsorbable) in a test meal. Analysis for polyethylene glycol in the samplings provided information as to dilution or concentration of the test meal.

Studies using the intubation techniques have provided information about the concentration of salts along the intestine (Karr and Abbott, 1935), the length of the intestinal tract (Hirsch *et al.*, 1956), the pH of the intestinal fluids (Borgström *et al.*, 1957), the effect of drugs on motility of the intestinal tract (Lorber and Shay, 1955 ; Higgins *et al.*, 1956), and the disappearance of various substances (sugar in particular) when placed in the lumen (Nicholson and Chornock, 1942 ; Bucher *et al.*, 1944 ; Cummins, 1952).

Scholer and Code (1954) have described a procedure for administering deuterium by mouth or by intestinal intubation and have measured the appearance of deuterium in successive samplings of arterial blood. With prior knowledge of the rate of disappearance of intravenously administered deuterium from arterial blood, one can estimate the unidirectional flux of water from the intestine to the blood. Using this technique, Higgins *et al.* (1957) demonstrated that the unidirectional flux of water and sodium from the small intestine was slower than normal in patients with nontropical sprue who had relapsed. The observations of Higgins *et al.* (1957) confirm earlier work of Newsholme and French (1954) who had placed Na^{24} into the duodenum of patients with sprue and measured the rate at which radioactivity rose in the hand by having their subjects clasp a tubular gamma-ray counter.

The measurements just cited are those of a unidirectional flux of sodium and water *out* of the intestinal lumen and were not concerned with the sodium and water which flowed *into* the lumen at the same time.

Although the net absorption or secretion of sodium and water is traditionally considered more important than unidirectional flux, these studies of unidirectional flux provide a fresh look at water and sodium transfers across the human intestine.

C. Surgical Preparations

Many types of permanent anatomical modifications of the intestine have been made, usually in the dog. In the most common procedure, a 15–30-cm length of duodenum, jejunum, ileum, or colon is resected and the continuity of the intestinal tract re-established. The resected portion is closed on one end and affixed to the peritoneal wall, the other end is left open and brought out through the skin, forming the stoma of a pouch into which solutions may be placed and samples withdrawn. This preparation, called a Thiry fistula (Thiry, 1865), has been modified often (Verzar, 1936). The most widely used modification was introduced by Vella (1881); here both ends of the resected segment of intestine are exteriorized and solutions may be perfused into one stoma, through the loop, and out the other stoma. Johnston (1932), Vaughan Williams (1954), and Pearson (1958) describe cannulae that can be fitted into the exteriorized ends of such segments to insure a leak-proof closure.

Employing a preparation similar to the Vella fistula with Krebs solution in the lumen, Cooperstein and Brockman (1959) have measured a potential of 10–40 mv across the dog colon, the lumen being negative; however no potential was found across the small intestine. Curran and Solomon (1957) found the lumen of the rat ileum to be positive (7–18 mv) with respect to the blood when the sodium concentration in solutions in the lumen was less than 100 meq/liter. The reasons for these differences are not yet apparent.

Thiry fistulas prepared by the writer in 1952 are still functioning in 1959. The fistulas have not deteriorated physiologically or histologically (Berger *et al.*, 1959a), although there has been criticism to this effect (Maggee, 1930; Verzar, 1936). One probable reason for their longevity is that irritating solutions were avoided, and only isotonic solutions were used in these fistulas.†

A second type of permanent surgical preparation is the valved fistula leading into the main intestinal tract; the valve prevents regurgitation of intestinal contents through the fistula (Basso, 1951). The valved fistula is a Roux-Y type of anastomosis, with the intussuscepted valve constructed in the antiperistaltic limb that leads to an exteriorized stoma. This fistula allows for sampling from the contents of the main intestinal tract and has provided an excellent preparation for the study of ions bound to exchange resins which have been fed by mouth (Field *et al.*, 1954).

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† Dennis (1940) has shown with histological sections that distilled water placed in the small intestine destroys the mucosa.

D. Vessel Cannulation

A number of studies have been conducted which involve injection of a given material into the arterial supply and/or sampling from the venous or lymphatic drainage of the intestine. The *in vitro* perfusion experiment has been applied to the rabbit colon by Garry *et al.* (1957). The arterial supply is cannulated and a venous effluent of up to 2–3 ml/min may be collected from the excised colon suspended in Krebs solution. The technique devised by Chinard (1955), which has been used so profitably to study the kidney, has been adapted for use with the intestine by Collings *et al.* (1958). It involves the rapid injection of test substances into the artery and collection of multiple blood samples from the venous drainage. A comparison of the concentration-time curves obtained for different substances leads to information about the distribution of those substances in the organ.

Sherman and Newman (1955) demonstrated functioning arteriovenous anastomoses in the stomach and duodenum by injecting glass microspheres, 42–425 microns (μ) in diameter, into the aorta and, a few seconds later, found spheres 100–180 μ in diameter, in the veins draining the duodenum (the average capillary diameter is 8–15 μ and maximum capillary dilatation is up to 30 μ). Grim and Lindseth (1958) injected glass microspheres, 20 μ in diameter and containing radiosodium, into an intestinal artery and measured their distribution in various tissues of the intestinal wall as an indication of the fractional blood flow to the various tissues.

Lymph ducts draining the intestine of the rat and dog have been cannulated with fine plastic catheters and lymph collected from the exteriorized end of the catheter after the animal had recovered from the surgical procedure (Bollman *et al.*, 1948, 1950; Grindlay *et al.*, 1950; Rampone, 1959). Benson *et al.* (1956) have demonstrated by this technique that deuterium and radiosodium are carried from the intestine to the general circulation almost entirely by way of the portal vein (99%) and little if any by way of the lymphatic system.

E. *In situ* and *in vitro* Preparations

In the anesthetized rat, sections of the intestine have been isolated *in situ* with blood supply intact. Wallace and Cushny (1898) and Budolfsen (1956) studied electrolyte and volume changes in solutions placed in such isolated segments. In similar experiments the two ends of the isolated segment may be cannulated *in situ* and solutions warmed to 37°C may be perfused through the segment, usually to be returned to the reservoir and recirculated (Fullerton and Parsons, 1956 ; Curran and Solomon, 1957). Hemoglobin, phenolsulfonphthalein, sodium sulfate, and creatinine have been used as volume indicators. In the *in situ* or *in vitro* preparations, it is necessary to maintain a favorable surface area-to-volume relationship, so that small net changes are not hidden in a large reservoir. Jacobs and Luper (1957) have described a system for the rat with a particularly small reservoir of 8 ml, the solution being circulated by a pump at a rate of 1 to 2 ml/min.

Fisher and Parsons (1949) described an *in vitro* preparation of the small intestine of a rat which has two closed circulations : one, through the lumen and the second, surrounding the serosa. A segment of small intestine is cannulated at either end, and an oxygen-saturated solution is circulated through the lumen prior to the interruption of the blood supply. The segment is then removed and mounted in a bath, i.e. the second circulation. Histological sections obtained after one hour were normal. Anoxia produced disturbances which far outlasted the period of anoxia.

Wiseman (1953) and Smyth and Whaler (1953a) modified the Fisher and Parsons procedure by circulating the luminal fluid through three segments suspended in a 25-ml bath. Smyth and Whaler (1953b) used *l*-alanine as a reference indicator to compare viability of one preparation with another. They started with equal concentrations of *l*-alanine on the mucosal and serosal sides, and related the transfer of ascorbic acid, phosphate, phenol red, and *p*-aminohippurate to the time required for the concentration of *l*-alanine on the serosal side to become twice that on the mucosal side. Later, Smyth and Taylor (1954, 1957) considerably improved the accuracy of the analysis of the transferred fluid by eliminating the outside circulation and allowing transferred fluid to drip from the serosal surface into a tube.

Wilson and Wiseman (1954) have described an *in vitro* preparation where a sac is prepared of the everted small intestine, fluid being placed on the serosal side (i.e. the inside) of the sac and the sac placed in a Warburg flask with Krebs phosphate or bicarbonate saline. This preparation will permit the study of respiration and transfer mechanism simultaneously. Schachter and Rosen (1959) using this technique have demonstrated that the proximal small intestine of rabbits, rats, and guinea pigs transfers calcium from mucosal to serosal surfaces against a concentration gradient and that prior vitamin D deprivation impairs this capacity for calcium transfer.

Excising the intestine and mounting it as a sheet between two chambers (Cooperstein and Hogben, 1959 ; Ussing and Andersen, 1955) has the advantage of isolating the intestine from extraneous factors. This

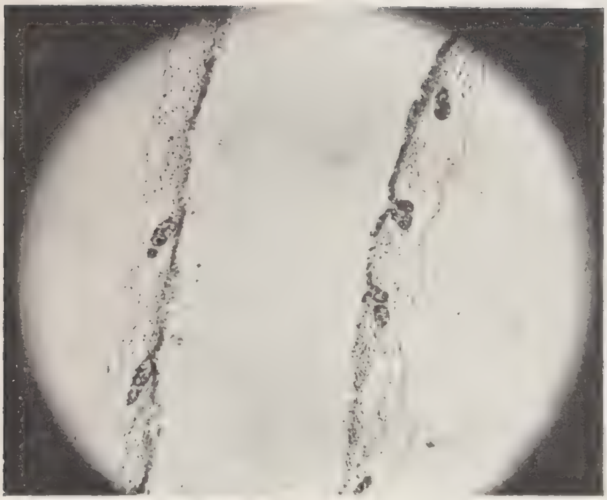

FIG. 1. This photomicrograph is taken from a histological section of the mucosa of a rabbit colon which had been stripped from the underlying muscle layers and mounted between two lucite chambers. After the experiment was completed, the sheet of mucosa, while still mounted between chambers, was fixed in formalin and then processed by the usual histological techniques. Note where mucosal cells are missing from the basement membrane.

type of preparation has yielded considerable information concerning the transport of electrolyte where frog skin or toad bladder is the membrane (Ussing, 1957; Leaf, 1958). When this preparation is applied to the intestine, however, it is of course necessary to distinguish between a membrane consisting of the full thickness of the intestinal wall and one consisting only of the mucosal cells and basement membrane. The mucosa of the rabbit colon can be peeled from the underlying tissue in a sheet, but there may or may not be mucosal cells covering the entire basement membrane (Fig. 1).

F. Comments on Methodology

Many studies have employed the material balance technique, and many others have reported what happens when various solutions are placed in the intestinal lumen. Such studies have been repeated again and again and are no longer likely to yield fresh information, except where new compounds or isotopes are used. Yet, the intestine offers a unique opportunity to study transport in the living mammal and to compare it with the *in vitro* results that have been obtained on squid axon, frog skin, and the like.

Studies of the transport of electrolytes in the living mammal usually have been confined to measuring the amount of material found in the excretions or secretions of a gland or organ. Such measurements have led to deductions concerning events in the cells forming the glands, as in the studies dealing with the kidney or the sweat gland (Berliner and Kennedy, 1948; Mudge *et al.*, 1948; Schwartz and Thaysen, 1956). The intestinal tract, and particularly the Thiry fistula, does not limit measurements to the net effect of a series of cells on the ultimate excretion, but, if one employs isotopically labeled materials, the simultaneous migrations of a particular molecule from lumen to blood and from blood to lumen may be measured across these cells in the living, active animal.

IV. Anatomy

The gross and histological structure of the intestinal tract are familiar to most (Maximow and Bloom, 1941). Two aspects of the anatomy of the intestinal tract deserve special mention. One is the appearance of the intestinal mucosa as seen through the electron microscope, and the other is an estimate of the surface area of the intestine per unit length.

The electron microscope reveals the free surface of the absorptive cells of the ileum of the adult rat to be composed of very minute processes projecting vertically into the lumen. The average length of the free part of the process is 0.62 μ, the average diameter is 0.08 μ. Granger and Baker (1950), estimate that these rodlike structures on the surface of the mucosal cell result in a 30-fold increase of the absorptive area of the rat intestine. Similar filamentous projections have been described on the free cell surface of the parietal, epithelial, and chief cells of the stomach, the epithelial cells of the gall bladder, cells of the thin limb of Henle's loop and the distal convoluted tubule of the kidney, the epididymis, and the choroid plexus (Dalton *et al.*, 1950, 1951; Dalton, 1951). Sjöstrand and Rhodin (1953), looking at these same projections in the mouse kidney, find them to be hollow ducts rather than rods and the density within the lumen to be the same as in the ground substance. All observers are agreed on the existence and size of these projections, but it has not been settled whether they are rods or channels.

The surface area is of interest because it is one basis for comparing the electrolyte flux across the intestine with that across some other tissue, for example, the frog skin. (Flux per gram of dry tissue could be another basis for comparison.) The mucosal area is considered that area obtained when the mucosal layer of cells is spread out in a longitudinal and transverse direction. Of course, this measurement, like the measurement of the area of the frog skin, neglects the appearance of the tissue as seen through the electron microscope. Warren (1939), Wood (1944), and Fisher and Parsons (1950) translated the serosal length to estimated mucosal surface area by rotometer measurements of the mucosal outline of projected microscopic sections. Measurements of the mucosal surface area per centimeter of serosal length are available for dog, cat, and rat intestine (Table I). In all probability the errors involved in estimating mucosal area from serosal length are within the variability of the measured flux rates.

TABLE I

ESTIMATED MUCOSAL SURFACE AREA PER LENGTH OF SEROSA

Species	Jejunum cm^2/cm	Ileum cm^2/cm	Colon cm^2/cm	References
Dog	54.0	40.0		Warren (1939)
Cat	49.5	35.5	5.6	Wood (1944)
Rat	8.5	5.1	2.2	Wood (1944)

V. Contents of the Intestinal Tract

The fluid contents of the intestine may constitute an appreciable portion of total body water in certain species (Table II). The intestinal tract contains 18% of the total water of the guinea pig. At the other extreme, the human intestine post mortem contains less than 1% of the total body water, sodium, potassium, and chloride.

TABLE II

WATER AND ELECTROLYTE CONTAINED IN THE GASTROINTESTINAL TRACT AS PER CENT OF THE TOTAL BODY POOL[a]

Species		Per cent of total body pool			
		Water	Sodium	Potassium	Chloride
Guinea pig	Cardia to anus	18.4			
Goat	Cardia to anus	16.3			
Rabbit	Cardia to anus	15.0			
Rabbit	Small intestine	2.0	3.2	1.2	2.5
Rabbit	Proximal colon	6.0	10.2	4.5	1.7
Hamster	Cardia to anus	11.9			
Mice	Cardia to anus	10.1			
Dogs	Cardia to anus	8.0			
Rats	Cardia to anus	6.7			
Man	Small intestine	0.7	0.7	0.4	0.7
Man	Proximal colon	0.3	0.4	0.4	0.3

[a] Data assembled from Cizek (1954) ; Edelman and Sweet (1956) ; Gotch *et al.* (1957) ; Nadell *et al.* (1956) ; Sweet *et al.* (1957).

A. Electrolyte Concentration in Intestinal Fluids

The concentration of electrolyte in the lumen of the small intestine in general tends to approximate an ultrafiltrate of plasma (Table III). In the ileum, however, chloride concentration tends to decrease compared with plasma, while the bicarbonate tends to increase (Visscher *et al.*, 1945 ; Visscher and Roepke, 1945 ; D'Agostino *et al.*, 1953). The pH of the intestinal contents of man increases from 6 in the duodenum to 8 in the ileum (Borgström *et al.*, 1957).

Using dogs with a fistula to the terminal ileum for sampling, Field *et al.* (1954) indicated that the sodium concentration in one set of hourly samples varied from 38 to 115 meq/liter, while the potassium concentration varied from 7.1 to 24.8 meq/liter. Despite such hourly variations, Field *et al.* were able to demonstrate clearly that reducing the dietary intake of sodium from 85 to 1 meq per 1250 calories was followed by a decrease in the sodium concentration in the ileum to 10–20 meq/liter and an increase in potassium to 80–110 meq/liter.

Wilson (1955) indicated that more sodium than chloride was lost through recent terminal ileostomies, the average concentrations being 123 and 68 meq/liter, respectively ; because the dietary intake usually contains equal amounts of sodium and chloride, a relative sodium deficiency may develop. Excretions from terminal ileostomies soon after their establishment contain considerably higher concentrations of sodium and chloride than older ileostomies. Crawford and Brooke

TABLE III

Electrolyte Concentrations in Intestinal Fluids

Species	Site of sampling	Sodium meq/liter	Potassium meq/liter	Chloride meq/liter	Bicarbonate meq/liter	References
Rabbit	Small intestine, at necropsy	100	39	58		Gotch *et al.* (1957)
Rabbit	Proximal colon, at necropsy	108	48	13		Gotch *et al.* (1957)
Dog	Jejunum, Thiry fistula	126–152	5–10	141–153	5–27	DeBeer *et al.* (1935)
Dog	Ileum, Thiry fistula	146–153	5	68–88	70–97	DeBeer *et al.* (1935)
Dog	Ileum, valved fistula, diet 85 meq sodium daily	121	14			Field *et al.* (1954)
Dog	Ileum, valved fistula, diet 1 meq sodium daily	10–20	80–115			Field *et al.* (1954)
Man	Small intestine, intubation	82–148	2–8	43–137		Lockwood and Randall (1949)
Man	Small intestine, intubation	123	7	122	8	Lesser and Pareira (1953)
Man	Small intestine, intubation adynamic ileus	48	3	40	5	Lesser and Pareira (1953)
Man	Ileostomy, recent	123		68		Wilson (1955)
Man	Ileostomy, recent	105–144	6–29	90–136		Lockwood and Randall (1949)
Man	Ileostomy, recent	200–400	5–10			Crawford and Brooke (1957)
Man	Ileostomy, adapted	46	3	21		Lockwood and Randall (1949)
Man	Ileostomy, adapted	100–200	10–20			Crawford and Brooke (1957)
Man	Cecostomy	52	8	42		Lockwood and Randall (1949)
Dog	Colon, Thiry fistula	136–151	6–9	60–88	86–93	DeBeer *et al.* (1935)
Dog	Formed stool, supernatant after centrifugation	25–49	11–22			Berger *et al.* (1959a)
Man	Formed stool, supernatant after centrifugation	22–31	80–132			Berger *et al.* (1959a)

(1957) report unusually high values for sodium in ileostomy fluids (200–400 meq/liter for recent, and 100–200 meq/liter for established ileostomies) compared to most other reports (Lockwood and Randall, 1949). The reason for the discrepancy is not evident, but all reports agree that with time the excretions of the terminal ileostomy approach the composition of normal stool.

B. Effect of Adrenalectomy and Drugs on Intestinal Contents

There is some evidence that the electrolyte content of the small intestine might be altered by the adrenal gland. Following administration of sodium chloride by mouth, more chloride is found in the intestine of the adrenalectomized rat than in the normal control (Clark, 1939). Dennis and Wood (1940) observed that two adrenalectomized dogs with ileal Thiry fistulas lost sodium into the intestinal lumen and this loss was corrected by the administration of adrenocortical extract.

The absorption of water from an isolated loop of dog jejunum is increased by atropine and Pitressin, and decreased by the mercuric ion. Atropine sulfate, injected subcutaneously, increases the absorption of water and chloride from the jejunum (Tidball and Tidball, 1958). In experiments where pressure was kept constant, the increase in absorption was attributed in part to an increase in surface area (Blickenstaff and Lewis, 1952). Pitressin in a dosage of 0.01 units/kg subcutaneously increases the absorption of water (Blickenstaff, 1954b). Mercuric chloride or meralluride (Mercuhydrin) placed in the jejunum reduces the absorption of sodium, chloride, and water (Blickenstaff, 1954a). Isotonic salt solutions placed in the isolated dog ileum tend to become hypotonic (Ingraham and Visscher, 1936b ; Visscher *et al.*, 1945) and this change in tonicity is prevented by mercuric chloride (Visscher and Roepke, 1945).

C. Ammonia in the Intestine

The absorption of intestinal ammonia is considered an etiological factor in episodic stupor which occurs in the course of cirrhosis of the liver. Kirk (1936) published an extensive monograph on amino acid and ammonium metabolism, which related the stupor to ammonia that had originated in the intestinal tract and that had reached the general circulation by bypassing the liver through the collateral circulation. There is still older literature on " Bild einer Vergiftung " or picture of poisoning following meat ingestion observed in Pavlov's laboratory in dogs with a fistula between the portal vein and inferior vena cava (Hahn *et al.*, 1893). The current interest in ammonia as the etiological agent in hepatic coma was sparked by the observation of stupor occurring after the administration of cation exchange resin in the ammonium cycle to patients with cirrhosis of the liver (Gabuzda *et al.*, 1952).

The ammonia-producing bacteria in the large intestine are one source of the ammonia (Silen *et al.*, 1955). Another is the amide nitrogen of glutamine and of other amino acids after protein feedings, this ammonia arising in the upper bowel independently of bacterial action (Webster *et al.*, 1958). A third source, which has not received much attention, is the intestinal mucosa itself. Ammonia appears in isotonic salt solutions placed in Thiry fistulas that are separate from the main stream of the gastrointestinal tract (Ingraham and Visscher, 1938 ; Bucher *et al.*, 1950). Herrin (1940) has demonstrated that the concentration of ammonia nitrogen in jejunal Thiry fistulas was 6–10 mg % in dogs on a carbohydrate diet, rising to 40–60 mg % when the animals were fed meat.

D. Urine in the Intestine after Ureterosigmoidostomy

The implantation of the ureters into the intestinal tract has been employed in the surgical treatment of lesions of the bladder. The first operation was performed by Simon (1852) who considered that " many of the animal creation excrete urine into the rectum " referring to the cloaca in birds. The presence of urine in the intestine following ureterosigmoidostomy has led to hyperchloremic acidosis (Ferris and Odel, 1950 ; Stamey, 1956). A net absorption of chloride and a net secretion of bicarbonate such as occurs in the ileum and colon of the dog (Ingraham and Visscher, 1936a ; Visscher *et al.*, 1945 ; Visscher and Roepke, 1945 ; D'Agostino *et al.*, 1953) contribute to the hyperchloremic acidosis, as does an excessive absorption of chloride over sodium such as occurs when urine is placed in the isolated dog colon (Pers, 1954).

In addition to hyperchloremic acidosis, patients with ureterosigmoidostomies develop potassium deficiency with time. The kidney is obliged to excrete an unusually high load of electrolyte, including sodium, as the electrolyte repeatedly makes the circuit from absorption in the colon to excretion by the kidney. A high renal load of sodium promotes potassium excretion by the kidney (Mudge, 1958), while only small amounts of potassium are absorbed by the colon (Pers, 1954). These circumstances eventually lead to potassium deficiency (Matern, 1954 ; Stamey, 1956 ; Mudge, 1958).

Following ureterosigmoidostomy, renal damage may occur secondary to ureteral obstruction or recurrent pyelonephritis from retrograde renal infection by colon bacteria. Renal damage is not necessary for hyperchloremic acidosis or potassium deficiency (Stamey produced this state in a normal man by repeated urine enemata), but in view of the high load of electrolyte placed on the kidney, it is not surprising that these disturbances occur almost exclusively in patients with reduced renal function (Lapides, 1951 ; Perry *et al.*, 1955).

VI. Fecal Excretion of Electrolyte

It still needs to be emphasized that electrolyte appearing in the stool is not simply the unabsorbed portion of the dietary intake. Sodium, potassium, chloride, and water in the intestinal lumen exchange with their respective total body pools, and the amounts appearing in the stool constitute a fraction of that pool. Hence, a sodium ion appearing in the stool is not necessarily an unabsorbed sodium ion that was ingested a day or so earlier. The circumstances with calcium, magnesium, phosphorus are more complicated, since only a portion of the dietary intake exchanges with the total body pool, but the principle of an exchange with total body pool still applies. The kinetics of fecal excretion of electrolyte is best considered in terms of a total exchangeable body pool with inflow via the diet and outflow via the stool.

Normal values for electrolyte excreted in the stool of man, dog, and rat are shown in Table IV. These values may be best discussed in relation to the factors that will change them. The writer has chosen to start the discussion with a consideration of the results obtained when cation exchange resins are given. These resins are insoluble, they have a negative charge, and cations adhere to their molecular surface.

A. Cation Exchange Resins

The ingestion of cation exchange resins will alter the electrolyte composition of the feces (Irwin *et al.*, 1949). Resins may be of some benefit in the treatment of selected patients with dropsy due to congestive heart failure or cirrhosis of the liver, as these patients fail to excrete sodium in the urine in amounts equal to the dietary intake. The dietary sodium that is retained does not turn body fluids to brine as body sodium concentration is maintained by an equivalent retention of water and this leads to the formation of dropsy. When cation exchange resins are fed, sodium is removed in the stool. This fecal sodium (or potassium) is almost certainly not the same ion that was fed in the diet, but is derived from the body pool of sodium and potassium (Spencer *et al.*, 1954). This is because there is a continuous exchange between the ions on the insoluble resin and the ions in the surrounding solution. If the composition of the intestinal fluid is altered by the intestinal mucosa, a new distribution of ions is established between the resin and the surrounding medium. The balance between the absorption of sodium by the colon and the "attraction" of the resin for the sodium eventually determines how much sodium is excreted in the stool (Berger and Steele, 1952).

TABLE IV

NORMAL VALUES FOR DIETARY INTAKE AND FECAL EXCRETION OF ELECTROLYTES IN RAT, DOG AND MAN[a]

Species	Sodium Dietary intake meq/day	 Fecal excretion	Potassium Dietary intake meq/day	 Fecal excretion	Calcium Dietary intake meq/day	 Fecal excretion	Magnesium Dietary intake meq/day	 Fecal excretion	Chloride Dietary intake meq/day	 Fecal excretion	Phosphorus Dietary intake mmole/day	 Fecal excretion
Rat[b]	0.5–5.0	0.1–0.3	1.0–5.0	0.4–0.7	1.0–5.0	0.5–3.0	1.0–2.0	0.8–1.5	0.5–5.0	0.1–0.3	2.0–6.0	0.5–2.0
Dog[c]	10–75	1–10	20–60	0.5–4.0	1–150[e]	3–140[e]	(1–15?)	(1–10?)	10–75	0.5–5.0	20–40	15–20
Man[d]	50–150	0.5–5.0	50–75	5–15	25–75	15–65	20–40	10–30	50–150	0.5–3.0	25–50	10–25

[a] These data were recalculated from reports in the literature, usually control data for various experimental procedures. The range was selected from the most frequently reported values. To convert milliequivalents or millimoles to milligrams, multiply by 23 for sodium, 39 for potassium, 20 for calcium, 12 for magnesium, 35.5 for chloride, and 31 for phosphorus.

[b] References for rat: Berger *et al.* (1951); Hegsted *et al.* (1951); Kjerulf-Jensen (1941); McChesney (1952); Miller (1926); Rottenstein (1938).

[c] References for dog: Barbour and Winter (1931); Danowski *et al.* (1951); Davis *et al.* (1952, 1953, 1959); Davis and Howell (1953); Field *et al.* (1955); Hoff-Jorgensen (1946); Liu and McCay (1953); Loeb *et al* (1933); Mendel and Benedict (1909a, b); Morgan (1934); Poutsiaka *et al.* (1957).

[d] References for man: Barnes *et al.* (1958); Blau *et al.* (1957); Dempsey *et al.* (1958); Dole *et al.* (1950); Irwin *et al.* (1949); Kjerulf-Jensen (1941); Leaf and Couter (1949); Macy (1943); Relman and Schwartz (1952); Schmidt and Greenberg (1935); Shohl (1939); Tibbetts and Aub (1937).

[e] Dogs fed lean meat receive minimal amounts of calcium (0.5–1.0 meq/100 gm lean meat) while certain commercial dog foods contain excessive amounts of calcium (e.g. Purina Laboratory Chow, Big Red Dog Food contains 65–80 meq/100 gm of food).

B. Hormonal Factors in Sodium and Potassium Excretion

Hormonal factors will influence the amount of sodium appearing in the lumen of the colon. This is not unexpected, but support for this observation has been scant until recently. Since 1951, however, evidence has accumulated to indicate that fecal excretion of sodium is decreased after the administration of mineralocorticoids and in the state of dropsy. It is increased when the adrenal gland is absent.

In man the fecal excretion of sodium is so small and variable that a decrease in excretion would be difficult to demonstrate ; however, a decrease could be demonstrated if a higher base line for fecal sodium excretion were established by feeding cation exchange resin. The administration of deoxycorticosterone acetate to man receiving cation exchange resin reduces the amount of sodium appearing in the stool and hence reduces the net absorption from the colon (Berger *et al.*, 1951). Less sodium is removed by the resin from the edematous patient who has an excess of sodium-retaining hormone (aldosterone) than from the normal individual (Berger and Steele, 1952). Administration of resin to individuals who lack the salt-retaining hormone (Addison's disease) results in a large excretion of sodium in the stool which again may be reduced by the administration of deoxycorticosterone (Emerson *et al.*, 1953). Duncan *et al.* (1956) demonstrated in man receiving cation exchange resin that the excretion of a large amount of fecal sodium was associated with a small amount of urinary aldosterone and the excretion of a small amount of fecal sodium was associated with a large amount of urinary aldosterone.

The rat normally excretes a sizable fraction of the dietary sodium in the stool, so that a decrease in fecal excretion following a drug or procedure may be documented without the use of cation exchange resin. After administration of deoxycorticosterone to the rat, fecal sodium decreases (Berger *et al.*, 1951). The administration of rather large doses (1 mg/kg/day) of 9α-fluorohydrocortisone to the dog decreases fecal excretion of sodium and increases fecal excretion of potassium (Poutsiaka *et al.*, 1957).† The large increase in fecal potassium excretion observed after 9α-fluorohydrocortisone in the dog was not observed after deoxycorticosterone administration to the rat (Berger *et al.*, 1951). The normal stool of rat and man contains more potassium than sodium, whereas the converse is true for the normal dog (Table IV).

During the development of ascites, following constriction of the thoracic inferior vena cava in the dog, the fecal output of sodium decreases and that of potassium increases (Davis and Howell, 1953).

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† In these experiments on dogs eating 42 meq of sodium per day, the control values for fecal sodium are quite high, 15 20 meq/day, compared to reported values of other observers (Table IV).

This decreased ratio of sodium to potassium in the feces was found to be correlated with increased aldosterone excretion in the urine (Davis *et al.*, 1959), and to return toward normal after adrenalectomy (Davis *et al.*, 1953).

The evidence cited here indicates that the fecal excretion of sodium in man, rat, and dog is at least in part regulated by the presence or absence of mineralocorticoids.

C. Divalent Cations

The excretion of calcium, phosphorus, and magnesium is covered extensively in other chapters of this treatise; hence comments about them here are limited. A portion of these divalent ions appearing in the stool represents unabsorbed dietary electrolyte, while part constitutes endogenous electrolyte added to the intestinal lumen by the bile, by pancreatic juice, and through the intestinal mucosa itself. DeBeer *et al.* (1935) report calcium, phosphorus, and magnesium in Thiry fistula secretions (note that the content of the Thiry fistula is not contaminated by secretions from other parts of the intestinal tract).

With the use of radioactive tracers the amount of divalent stool electrolyte which is endogenous and that portion which is exogenous has been calculated for phosphorus excretion in man and rat (Kjerulf-Jensen, 1941) and for calcium excretion in cattle and man (Comar *et al.*, 1953; Blau *et al.*, 1954; Bronner and Harris, 1956). In man on a constant calcium intake, one week after the oral administration of a single dose of Ca^{45}, the specific activities of the plasma and urine are equal and regularly exceed the specific activity of fecal calcium. The ratio of the specific acitivity of calcium in stool to that in plasma or urine indicates the fraction of stool calcium which is endogenous. Blau *et al.*, (1954, 1957) demonstrated the endogenous fecal calcium to be about 5 meq/day and independent of the level of calcium intake. In man on an ordinary mixed diet, Kjerulf-Jensen (1941) demonstrated the endogenous fecal phosphorus to be about 3.5 mmole/day.

Fecal excretion of calcium is increased in hyperthyroidism and in Vitamin A deficiency (Tibbetts *et al.*, 1932). The fecal excretion of calcium is probably not affected to any significant degree by the parathyroid hormone (see Vol. I, Chapters 11 and 14).

VII. Mechanisms of Absorption and Secretion

A change in the amount of sodium, potassium, chloride, and water in the intestinal lumen is not the consequence of a one-way passage into or out of the intestine. Rather, it is the consequence of two simultaneous fluxes across the intestinal mucosa, one from the blood to the lumen, the other from lumen to blood.

A. Measurement of Unidirectional Flux Rates

Unidirectional flux rates are measured with isotopic tracers, but the mathematical analysis for a system such as exists with a Thiry fistula has received scant attention (Goldwater and Stetten, 1947 ; Sheppard and Beyl, 1951 ; Lax and Wrenshall, 1953 ; Hart, 1957). Tracers have been commonly used in systems in the state of " dynamic equilibrium " (Schoenheimer, 1942 ; Feller *et al.*, 1950 ; Robertson, 1957). This state involves two assumptions : (*a*) that the size of the metabolic pool does not change during the period of observation, i.e., the rates of material entering (inflow) and leaving the pool (outflow) are equal and (*b*) that the rates of flux are constant during the period of observation. The mathematical methods necessary for the calculation of flux rates in this type of system are well established.

In many biological systems, however, there is a net change with time ; in fact, these were the only systems the kinetics of which could be studied prior to the availability of isotopes. Although there are many types of such " nonsteady-state " systems, the type treated here applies to the Thiry fistula and is one where the rates of inflow and outflow between the compartments, although unequal, are constant with time (Berger and Steele, 1958).

The assumptions necessary to develop these equations are (*a*) complete and instantaneous mixing in either compartment and (*b*) the transport mechanism involved does not fractionate the isotopes. The condition of equal inflow and outflow between compartments, i.e., " the steady state " or " dynamic equilibrium," then becomes a special case of the general equations.

	Time	Compartment I	Compartment II
Amount of material (Labeled plus unlabeled)	0	A_0	B_0
Specific activity		θ_{A0}	θ_{B0}
Exchange from I to II		$\xrightarrow{\quad\alpha\quad}$	
Exchange from II to I		$\xleftarrow{\quad\beta\quad}$	
Amount of material		$A = A_0 + (\beta - \alpha)t$	$B = B_0 + (\alpha - \beta)t$
Specific activity		θ_{A_0}	θ_{B_0}
Exchange of material		$\xrightarrow{\alpha \Delta t}$ $\xleftarrow{\beta \Delta t}$	
Exchange of tracer		$\xrightarrow{\theta_A \alpha \Delta t}$ $\xleftarrow{\theta_B \beta \Delta t}$	

FIG. 2. Notations used in mathematical formulations.

The following notations are introduced (Fig. 2). A represents the amount of material (labeled plus unlabeled) in Compartment I; B represents the amount of material in Compartment II; θ_A represents the specific activity (the ratio of labeled material to labeled plus unlabeled material) of the material in Compartment I; θ_B represents the specific activity in Compartment II; α is the rate of passage of material from Compartment I to Compartment II (amount per unit time); β is the rate of passage of material from II to I; t represents time.

The initial conditions at $t=0$ are

$$A=A_0 \tag{1a}$$

$$B=B_0 \tag{1b}$$

$$\theta_A=\theta_{A_0} \tag{1c}$$

$$\theta_B=\theta_{B_0} \tag{1d}$$

The mass of material (labeled plus unlabeled) in Compartment I at time t is

$$A=A_0+(\beta-\alpha)t \tag{2}$$

The mass of tracer in Compartment I at time t is $\theta_A[A_0+(\beta-\alpha)t]$. The mass of material at time $t+\Delta t$ is $A_0+(\beta-\alpha)(t+\Delta t)$. The mass of tracer at $t+\Delta t$ becomes $\theta_A[A_0+(\beta-\alpha)t]+\theta_B\beta\Delta t-\theta_A\alpha\Delta t$. The specific activity in Compartment I at $t+\Delta t$ is $(\theta_A+\Delta\theta_A)$ and equal to

$$\frac{\theta_A[A_0+(\beta-\alpha)t]+\theta_B\beta\Delta t-\theta_A\alpha\Delta t}{A_0+(\beta-\alpha)(t+\Delta t)},$$

which may be rearranged to

$$\frac{\Delta\theta_A}{\Delta t}=\frac{\beta(\theta_B-\theta_A)}{A_0+(\beta-\alpha)(t+\Delta t)} \tag{3}$$

For $\Delta t\to 0$,

$$\frac{d\theta_A}{dt}=\frac{-\beta(\theta_A-\theta_B)}{A_0+(\beta-\alpha)t} \tag{4}$$

Similarly for Compartment II,

$$\frac{d\theta_B}{dt}=\frac{\alpha(\theta_A-\theta_B)}{B_0+(\alpha-\beta)t} \tag{5}$$

Eq. (5) is subtracted from Eq. (4) to yield

$$\frac{d(\theta_A-\theta_B)}{(\theta_A-\theta_B)}=-\frac{\beta\,dt}{A_0+(\beta-\alpha)t}-\frac{\alpha\,dt}{B_0+(\alpha-\beta)t} \tag{6}$$

Eq. (6) may be integrated to yield

$$\ln\frac{\theta_A-\theta_B}{\theta_{A_0}-\theta_{B_0}}=\frac{\beta}{\alpha-\beta}\ln\frac{A}{A_0}-\frac{\alpha}{\alpha-\beta}\ln\frac{B_0}{B} \tag{7}$$

With Eq. (2), Eq. (7) may be rearranged to

$$\beta = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}}\right] \tag{8}$$

and

$$\alpha = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}} - 1\right] \tag{9}$$

The condition $\alpha \neq \beta$ is necessary for the solution of Eq. (7). For the special case in which $\alpha = \beta$ the differential equation has the form

$$\frac{d(\theta_A - \theta_B)}{(\theta_A - \theta_B)} = -\left[\frac{\beta}{A_0} + \frac{\alpha}{B_0}\right] dt \tag{10}$$

the solution of which is

$$\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} = -\left[\frac{\beta}{A_0} + \frac{\alpha}{B_0}\right] t \tag{11}$$

and

$$\beta = \alpha = \frac{-\ln\dfrac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}}}{\left[\dfrac{1}{A_0} + \dfrac{1}{B_0}\right] t} \tag{12}$$

Eqs. (8) and (9) have general application to the nonsteady state in which rates of flow between two compartments are unequal but constant with time. Eq. (12) applies to the special case in which rates of flow between compartments are equal. Transfer rates (α and β) are in terms of amount of material per unit time and are determined from measurements of specific activity (θ_A, θ_B) and compartment size (A, B).

The equations which apply to the general case may, of course, be further modified for the particular experimental circumstances. For the measurement of the transfer of sodium for instance, into and out of an isolated loop of bowel, mathematical considerations indicate that complete mixing in Compartment II (the extraluminal sodium space) need not be assumed and the assumption of complete and instantaneous mixing need be made only for Compartment I (the intraluminal sodium). In addition, the effects of sampling must be considered in arriving at values for inflow and outflow. When methyl cellulose is used as an indicator of the volume of fluid in the lumen it may be shown (Berger and Steele, 1958) that

$$\beta = \frac{A - A_0}{t} \left[\frac{\ln [(\theta^n_A - \theta^n_B)/(\theta_{A_1} - \theta_{B_1})]}{-\ln \dfrac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} \right] \qquad (13)$$

and

$$\alpha = \frac{A - A_0}{t} \left[\frac{\ln [(\theta^n_A - \theta^n_B)/(\theta_{A_1} - \theta_{B_1})]}{-\ln \dfrac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} - 1 \right] \qquad (14)$$

The rate of transfer of sodium into the bowel in microequivalents per minute is β; the rate out of the bowel is α: the number of microequivalents of sodium in the bowel, corrected for that sodium previously removed in sampling, is A_0 at the beginning and A at the end of an observation period of t minutes duration; θ_{A_1}, $[\mathrm{Na}]_1$ and $[\mathrm{MC}]_1$ are, respectively, the specific activity (cpm/μeq), sodium concentration (μeq/ml), and methyl cellulose concentration (mg/ml) in the first sample, and θ^n_A, $[\mathrm{Na}]^n$, and $[\mathrm{MC}]^n$ are their values at any subsequent sampling from the bowel lumen. Plasma specific activity is θ_{B_1} at the first, and θ^n_B at any subsequent sampling.†

B. Unidirectional Flux for Various Tissues

Values for the *in vivo* unidirectional transfers of sodium, potassium, chloride, and water across the small intestine (Visscher *et al.*, 1944a, b; Curran and Solomon, 1957; Berger *et al.*, 1959a, b) may be converted to amount per unit surface area per unit time to compare with unidirectional transfers across other tissues *in vitro* (Table V). In general, the unidirectional flows of sodium, potassium, chloride, and water are of the same order of magnitude across the small intestine as across other tissues for which data are available. The one exception is the erythrocyte.

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† In calculations of the unidirectional transfer of sodium, into and out of isolated loops of intestine, Visscher *et al.* (1944b) used the decrease in the total number of labeled ions as a measure of the flux out of the bowel. Dividing the total number of labeled ions lost by the mean specific activity is a measure of the outflow of sodium from the bowel lumen [see Vol. I, Chapter 5, Eq. (25)]. The difficulty, however, lies in estimating the mean specific activity. Visscher used the arithmetic mean, whereas the correct mean is a function of (*a*) the integral of the change in specific activity and (*b*) the effects of the net change. With decreases in specific activity of sodium up to 40%, there is little difference between the arithmetic and correct means. With larger changes in specific activity, the arithmetic mean overestimates the correct mean at a rapidly increasing rate

where the sodium and potassium fluxes are considerably slower than in other tissues; however, the chloride flux across the erythrocyte is probably in the same range as the other tissues.†

The ratio of water molecules to sodium ions in transit through a membrane may be compared to their ratio prior to transit in the solution bathing the membrane. Surface area is no longer a necessary measurement when considering ratios of water to sodium, and such data do not suffer the inaccuracies attendant upon estimates of transfer rates per unit surface area. There are situations where water moves considerably faster than sodium. Frog skin, *in vitro*, transfers water into the frog 50 times as fast as sodium (Garby and Linderholm, 1953). Flexner and Gellhorn (1942) have indicated that water is exchanged 50 times more rapidly than sodium in the amniotic fluid of the pregnant guinea pig. In the case of the dog small intestine, however, water enters and leaves the lumen only twice as fast as sodium (Berger *et al.*, 1959a). This ratio is of the same order as their free diffusion coefficients (at 37°C, 3.03×10^{-5} cm^2 sec^{-1} for deuterium, 1.80×10^{-5} cm^2 sec^{-1} for sodium [Garby, 1957]).

C. Drug Effects on Unidirectional Flux

1. *Deoxycorticosterone*

The excretion of sodium by the colon, the kidney, the sweat gland, and the salivary gland is under hormonal control (Berger *et al.*, 1951; Berger and Steele, 1952; Conn, 1949; White *et al.*, 1955). To effect conservation of sodium, its absorption from the lumen of the nephron or colon to the blood is increased, while its secretion from the blood to the lumen of a sweat or salivary gland is decreased. It would seem that sodium transfer would be affected in the same manner across all four membranes. The possibility exists that for the four tissues, absorption or secretion, i.e., net transfer, may be the consequence of two unidirectional flows, one entering and the other leaving the lumen, the balance of these two flows determining the amount of sodium appearing in the stool, urine, sweat, and saliva. Because the amount of sodium appearing in the intestinal lumen is known to be the net result of two flows across the mucosa, one from blood to lumen, the other from lumen to blood, the intestine provides a means for deciding which of the unidirectional flows is altered by deoxycorticosterone.

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† The chloride flux across red cells is so rapid that the specific activities of intracellular chloride and the chloride in the surrounding medium are equal in a matter of seconds, thus creating considerable difficulties in measurement of flux rates (Love and Burch, 1953). Tosteson (1956) used an ingenious flow tube and estimated the rate constant for chloride outflux from beef red cells to be 10^6 times the rate constant for potassium influx.

TABLE V

Unidirectional Fluxes of Sodium, Potassium, Chloride, and Water for a Number o Tissues

Substance	Species	Tissues	Flux into animal	Flux out of animal	References
SODIUM	*In vitro*		$\mu\mu$moles cm^{-2} sec^{-1}	$\mu\mu$moles cm^{-2} sec^{-1}	
	Frog	Skin	200–1300	20–120	1
	Toad	Bladder	70–1300	20–700	2
	Man	Erythrocyte	0.05	0.05	3
	Toad	Large intestine	370–700	130–280	4
	Guinea pig	Cecum	1300–2600	900–2000	5
	In vivo				
	Necturus	Proximal nephron	200–1200	266–(1200)[b]	6
	Rat	Ileum	3400	2400	7
	Dog	Ileum	620–3100	300–3000	8
POTASSIUM	*In vitro*		$\mu\mu$moles cm^{-2} sec^{-1}	$\mu\mu$moles cm^{-2} sec^{-1}	
	Squid	Giant axon	15–30	20–40	9
	Dog	Erythrocyte	0.002	(0.002)[b]	10
	Man	Erythrocyte	0.033	0.031	11
	Frog	Gastric mucosa	100–360	10–30	12
	In vivo				
	Dog	Small intestine	20–80	40–90	13
CHLORIDE	*In vitro*		$\mu\mu$moles cm^{-2} sec^{-1}	$\mu\mu$moles cm^{-2} sec^{-1}	
	Frog	Skin	140–350	30–100	14
	Frog	Gastric mucosa	750–2400	670–2400	15
	In vivo				
	Rat	Ileum	2000	730	16
	Dog	Ileum	1100–4300	0–3150	17
WATER	*In vitro*		mμliters cm^{-2} sec^{-1}	mμliters cm^{-2} sec^{-1}	
	Frog	Skin	60–100	60–100	18
	Toad	Skin	100–120	100–120	19
	Frog	Gastric mucosa	400	400	20
	In vivo				
	Rat	Jejenum-ileum	10	10	21
	Dog	Jejunum	17–27	13–17	22
	Dog	Ileum	15–45	20–55	23

[a] References:

1. Linderholm (1952); Ussing (1957).
2. Leaf *et al.* (1958).
3. Sheppard and Beyl (1951); Solomon (1952); Streeten and Solomon (1954).
4. Ussing (1957).
5. Ussing and Andersen (1955).
6. Solomon *et al.* (1957); Oken *et al.* (1959.)
7. Curran and Solomon (1957).
8. Visscher *et al.* (1944b); Berger *et al.* (1959a).
9. Hodgkin and Keynes (1955a, b)
10. Frazier *et al.* (1954).
11. Solomon (1952); Streeten and Solomon (1954).
12. Crane and Davies (1949).
13. Berger *et al.* (1959b).
14. Linderholm (1952).
15. Hogben (1955); Heinz and Durb (1957).
16. Curran and Solomon (1957).
17. Visscher *et al.* (1944a).
18. Hevesy *et al.* (1935); Garby and Lind holm (1953); Koefoed-Johnsen an Ussing (1953).
19. Koefoed-Johnsen and Ussing (1953).
20. Hogben (1955).
21. Schanker *et al.* (1958).
22. Berger *et al.* (1959a).
23. Visscher *et al.* (1944a); Berger *et al.* (1959

[b] Values in parentheses are inferred from reported observations.

Studies on the large intestine reveal a decrease in the amount of sodium and an increase in the amount of potassium in the colonic lumen after administration of deoxycorticosterone. Detailed analysis with isotopes indicated that sodium transfer into the lumen was unchanged, while sodium transfer out of the lumen increased (Berger, 1959). Potassium transfers into and out of the colonic lumen were both increased by deoxycorticosterone ; the rate of potassium influx into the colon increased to a greater degree than its rate of outflux, thus resulting in a net increase of potassium in the colon (Berger, 1959).

2. *Organomercurials*

Mersalyl (Salyrgan) has also been studied for its effect on sodium transfer across the intestine (Berger, unpublished observations). Organomercurials are believed to reduce the transfer of sodium from the lumen of the renal tubule to the blood, but this was not found to be true for sodium flux across the dog colon. When 1 ml of Salyrgan was administered intravenously, the outflow of sodium from the colonic lumen was unchanged (in fact, increased slightly) but a large inflow of sodium into lumen ensued. Normally, the colon may be considered to inhibit the influx of sodium into the lumen ; apparently the administration of the mercurial compound permits sodium to pour into the colonic lumen.

VIII. Functional Characteristics of the Intestinal Mucosa

The intestinal mucosa is one of the more active metabolic tissues of the body. The oxygen uptake of mucosa scraped from the rat duodenum is 13–16 μl/mg of dry tissue per hour (Vitale *et al.*, 1954), a value at least equal to that of liver (10 μl/mg/hr) and considerably higher than for muscle (2 μl/mg/hr) (Hawk *et al.*, 1947).

The mucosa of the intestine has been credited with the ability of rapid regeneration, as evidenced by the large number of mitotic figures seen in histological sections. The renewal time has been estimated as 1–2 days for the mucosa of the rat (Leblond and Stevens, 1948) and 2–3 days for the mucosa of the cat (McMinn, 1954).[†] Practically all of the mitoses occur in the crypts of the mucosa and the newly formed cells migrate along the side of the villus to be extruded at the villus tip

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[†] The administration of colchicine arrests mitosis in the metaphase and renewal time may be estimated from the number of metaphase mitotic figures accumulated in a given period. In the rat, for example, the percentage of ileal mucosal cells in mitosis increases from 3.3 to 17.6% 6 hours after subcutaneous colchicine, hence 5.3 mitotic cycles occurred in 6 hours, or the average mitosis lasted 1.1 hours. Knowing that 3.3% of mucosal cells are in mitosis normally, and each mitosis lasts 1.1 hours, 100% of the cells would be replaced in 33 hours (Leblond and Stevens, 1948).

(Leblond and Stevens, 1948). By labeling the rat or mouse with tritiated thymidine, Sherman *et al.* (1959) have shown that the cells move from the base to the tip at a speed of about one cell width per hour. In the cat, the epithelialization of a small (1 cm^2) artificially denuded area occurs with cells which originate from the crypts near the ulcer margin and advance over the denuded area.† The advancing mucosa does not contain mitotic figures, but invaginations of this epithelial sheet into the underlying granulation tissue gradually deepen to form crypts and by the seventh postoperative day, mitoses are seen in the newly formed crypts. The rate of regeneration at the ulcer margin appears to remain the same as it was prior to the production of the gap, as shown by experiments with colchicine (McMinn and Mitchell, 1954).

Experiments conducted both *in vitro* and *in situ* showed that net water transfer is dependent on temperature, oxygen, glucose, and metabolic inhibitors such as phlorizin, monoiodoacetate, and substituted phenols (Fisher, 1955 ; Smyth and Taylor, 1957 ; Tidball *et al.*, 1957). In isolated rat ileum, unidirectional flux of sodium and chloride from mucosa to serosa is reduced approximately 75% by the absence of glucose in the perfusing solutions (flux from serosa to mucosa is somewhat reduced) (Curran and Solomon, 1958). These observations suggest that water and salt transfer is in part dependent on the maintenance of an active metabolizing mucosa for which glucose is nutritive.

These functional and biochemical characteristics have, in the last analysis, a structural basis. One view of a cell boundary (Collander, 1937 ; Höber, 1945 ; Davson and Danielli, 1952 ; Ussing, 1954) is that most of it consists of lipoid material through which lipoid-soluble molecules pass. This concept is based on observations of the rates at which foreign compounds enter plant cells, erythrocytes, and sea urchin eggs ; the greater the lipoid solubility of the compound, the faster the penetration. A much smaller portion of the boundary consists of water-filled pores in the lipoid material through which small ions and water pass. The pores are sufficiently small in size to prevent the penetration of large water-soluble molecules. Based on Fick's law of diffusion, and Poiseuille's law of laminar flow, and assuming an ideal membrane with uniform cylindrical pores, the radius of these pores in the capillary wall has been estimated as 30–45 Å (Pappenheimer *et al.*, 1951 ; Pappenheimer, 1953). Assuming that what is true for the capillary is also true for the intestine, the equivalent pore radius for the rat ileum has been estimated as 36 Å (Curran and Solomon, 1957 ; Durbin *et al.*, 1958). Other measurements of interest with respect to the estimated pore radius are (*a*) the hydration radius of sodium (8 Å) (Dean, 1948) and (*b*) the average radius (400Å) of the processes projecting into the intestinal lumen, as seen through the electron microscope (Granger and Baker, 1950).

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† Larger areas of mucosa, 15 cm. in length, removed from the descending colon of the dog, do not regenerate even after 21 weeks (Hightower, 1958).

Schanker *et al.* (1958) and Hogben *et al.* (1959) present observations of the behavior of the lumenal boundary of the mucosa of the small intestine which conform to this structural picture of a lipoid membrane with pores. They studied the absorption rates of a variety of drugs and found that their rate of absorption of these organic acids and bases was directly related to the un-ionized, lipoid-soluble form of the drug. A consideration of the pK_a of the drug and its concentration in the plasma and in the intestinal lumen further characterized the mucosal boundary to have a "microclimate" at the site of absorption with a pH of about 5.3.

Schanker *et al.* (1958) also demonstrated that lipoid-soluble drugs like phenylbutazone and thiopental are absorbed through the rat intestine faster ($>50\%$) than deuterium oxide (41%) which has a much smaller molecular weight and is polar. The observation that lipoid-soluble drugs are transferred faster than water is compatible with the notion that a much larger area of the mucosal boundary is made up of lipoid material than of pores.

Once electrolyte and water pass the luminal boundary of the mucosa, do they diffuse to the capillary surface through the entire cell, do they stream through the cell cytoplasm in channels, or do they pass through the intercellular space, etc.? Grim *et al.* (1955) placed deuterium in the lumen of the dog ileum and then measured deuterium concentration in mucosa, muscle, and mesenteric venous blood sampled at intervals thereafter. The deuterium concentration approached an equilibrium value at a rate which was considerably slower in the mucosal cell than in either muscle or venous blood. One of the several possible interpretations of this observation is that water passes through the intestinal mucosa in a channel, either intra- or intercellular or both, and undergoes little mixing with the cytoplasm.

With the electron microscope, Weiss (1955b) demonstrated the development of granules in the mitochondria of the duodenal absorptive cell of the mouse, when large amounts of sodium or potassium chloride were administered orally. (Similar granules were seen in the mitochondria of the renal tubule cell.) He considers these granules to represent a segregation form for cations transported through the cell, analagous to an ion exchange resin, which would suggest passage through the cell. Weiss (1955a) has further demonstrated by electron microscopy the unusual pathway for fat through the mucosal cell. Lipid first passes through the apical membrane in particles less than 40 Å in diameter. These are then transformed into larger fat droplets which pass through the cytoplasm to the lateral wall of the cell which they penetrate to enter the intercellular space, then downward through the basement membrane to the extracellular space and into the lacteal. The anatomical pathway of water and electrolyte through the intestinal mucosa is entirely unknown at present.

There is evidence that unidirectional flows of sodium across the mucosa do not interfere with each other. Visscher *et al.* (1944b) observed "that the apparent permeability of the small gut to sodium ion entering from the blood is positively correlated with the rate at which the univalent cation . . . is leaving the gut." These observations would speak against interference of influx with outflux and would suggest that flow across the mucosa would be analogous to sodium, rather than potassium, flux across the membrane of the sepia axon. In the case of sodium, Hodgkin and Keynes (1955b) assumed that "the number of sites occupied in the sodium channels is sufficiently small for the fluxes to be nearly independent," while in the case of potassium they believe that potassium ions enter and leave the sepia axon in single file, making it difficult for external potassium ions to enter in the face of a predominant stream coming in the opposite direction.

The writer's current notion is that for each ion (sodium or potassium) that enters the luminal side of the mucosa, a corresponding ion leaves the capillary side, like a string of beads pulled through the mucosa. This would also hold for ions traveling from capillary to lumen. The intra- or intercellular composition of the mucosa is not altered, regardless of how fast the string of beads is pulled through. The passage of electrolyte through the mucosal cell and/or between the cells is visualized as two simultaneous chainlike streams, one passing from blood capillary to lumen, the other passing from lumen to blood.†

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† These streams may be related to each other. There is a high degree of correlation ($r = 0.92$) between the net flux and the ln of the ratio of the individual fluxes. This is true for sodium, potassium, and water in the small and large intestine of the dog (Berger, unpublished observations).

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CHAPTER 9

Renal Excretory Mechanisms

MACKENZIE WALSER AND GILBERT H. MUDGE

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I. Introduction

This chapter considers the intrinsic renal mechanisms which influence the urinary excretion of minerals, acid and water. Emphasis will be given both to the manner in which excretion is affected by plasma composition and to the mechanisms by which various discrete renal processes are regulated. The reader is referred to other chapters for a discussion of the renal contribution to the overall regulation of mineral balance. Other aspects of renal physiology are presented in the recent reviews by Smith (1956), Bradley (1957), and Winton (1956).

A. Definition of Terms

The four major processes by which the mammalian kidney elaborates urine may be briefly defined.

1. *Glomerular filtration*

Glomerular filtration is the formation of an ultrafiltrate of plasma that is virtually protein-free. Its composition has been established by direct analyses of samples obtained by micropuncture (Richards, 1934). The filtrate is formed across the semipermeable capillaries of the glomerular tuft by energy derived from the heart and transmitted to the kidney as arterial pressure.

2. *Tubular reabsorption*

Tubular reabsorption is the transport either of water or solute from the glomerular filtrate across the epithelium of the tubule to the peritubular space and thence into the vascular system. This concept of reabsorption simply defines the direction of net movement at the tubular site and is without implication as to the magnitude or nature of the process. Indeed, the characteristics of individual reabsorptive mechanisms vary greatly, depending upon the particular substance under consideration. For example, although both glucose and water are reabsorbed by the proximal portion of the tubule, the mechanisms are very different. Glucose reabsorption depends on cellular metabolism and can be inhibited by specific agents, while water reabsorption in this segment apparently occurs by simple diffusion.

3. *Tubular secretion*

Tubular secretion is net transport from the tubular epithelium into the tubular urine. Thus substances that are secreted may appear in the urine without having been filtered at the glomerulus. In most instances, this involves transport of plasma constituents from peritubular capillaries to urine; a few substances are formed within the tubular cells and conveyed directly to the urine. For example, urinary ammonia is derived from amino acids metabolized within the kidney cells. It should be emphasized that the term secretion defines the direction of net movement and, as in the case of reabsorption, does not imply the nature of the underlying mechanism.

A further distinction must be made between secretion, as used herein, and exchange of a single ionic species. If labeled sodium ions from the peritubular fluid were to exchange with unlabeled sodium ions from the tubular urine, analysis of the urine would suggest that the labeled sodium ions had been secreted by the tubule. However, secretion of sodium, as here defined, would require the net transport of sodium ions from peritubular fluid to tubular urine. Although evidence has been obtained that labeled ions of both sodium and potassium may enter the urine from the peritubular fluid, it is not certain whether these findings represent secretion or exchange of labeled for unlabeled ions (Chinard and Enns, 1955 ; Black and Emery, 1957).

4. *Excretion*

The term *excretion* will be used to refer to the final urine.

B. Historical Development

Certain features of renal electrolyte metabolism may be surveyed best against a brief historical background.

1. *Threshold*

When quantitative chemical methods were first employed, attention was focused mainly upon the concentration of minerals in the plasma or urine, and the term renal *threshold* was introduced to denote the plasma concentration of a given substance above which it is excreted in the urine and below which its rate of excretion is negligibly small. The term implies that such a renal mechanism is attuned to the concentration of the substance in the plasma independently of other parameters. It was soon recognized, however, that this single parameter was inadequate.

2. *Clearance*

Based on the early studies of urea and creatinine excretion, the clearance of a substance was defined as $C = UV/P$ in which C is clearance per unit time (usually one minute), U and P are urinary and plasma concentrations, respectively, and V is urine volume per unit time. The clearance is thus equal to a volume of plasma which contains the amount of the substance excreted during a given interval of time. A clearance can be calculated for any substance which can be quantified in both plasma and urine.

The clearance method has greatly increased the number of renal processes which can be analyzed quantitatively. By the selection of appropriate reference substances, it has become possible to measure the rate of glomerular filtration (usually as the inulin clearance in man, and as the inulin or exogenous creatinine clearance in the dog) and the rate of renal plasma flow (generally as the clearance of *p*-aminohippurate). The reader is referred to Smith's monograph (1951) for the physiological basis of these measurements. By their use it has been possible to estimate the amount filtered of any solute as the product of the glomerular filtration rate GFR and the plasma concentration P, corrected as necessary for plasma water, protein-binding, and the Donnan factor. The amount of the solute excreted per unit time can be measured directly. Hence, with a substance such as sodium that is thought to be filtered and reabsorbed, the amount reabsorbed per unit time can be calculated.

3. *Transport maximum* T_m

In the case of some substances, measurements of the rate of reabsorption have led to the formulation of a basic concept of tubular function, namely, that there are maximal rates of transport T_m which are presumed to be limited by intrinsic cellular components (Shannon *et al.*, 1941). Although acceptable T_m values have been measured for a variety of organic substances (see Smith, 1951), in the case of electrolytes, considerable difficulty has been encountered in attempting to evaluate mechanisms of transport in these terms.

The quantitative analysis of sodium reabsorption presents a particularly difficult problem. Under normal circumstances only a small fraction of the filtered sodium is excreted in the urine. By subtracting the amount excreted UV, which is small, from the amount filtered $P \times GFR$, which is large, one obtains the amount reabsorbed T, also large. Under a variety of experimental conditions T is largely determined by the value of $P \times GFR$. A plot of the amount reabsorbed against the amount filtered therefore depicts an almost linear function, even when considerable changes occur in the amount excreted. Thus the over-all rate of sodium reabsorption does not appear to be limited by a maximal value, or T_m.

Attempts to apply modifications of the T_m concept to electrolyte reabsorption have followed several lines. One proposal (see Wesson, 1957) is that sodium reabsorption is limited by a T_m only in the distal tubule ; in the proximal tubule reabsorptive transport is a constant fraction of the amount of sodium filtered. Total sodium reabsorption in this scheme would exhibit features intermediate between these two types of tubular function. While certain data consistent with this scheme have been presented, the postulates on which it is based are not subject to experimental verification at present.

Another modification of the T_m concept is the expression of maximal reabsorption per unit volume of filtrate rather than per unit time (Pitts and Lotspeich, 1946). Under certain experimental conditions this calculation yields a constant value for reabsorption of a number of inorganic ions despite moderate variations in the amounts filtered. The relationship implies that the rate of tubular transport varies with the filtration rate. For example, bicarbonate reabsorption expressed in this way is unaffected by day to day variations in filtration rate which result from changes in dietary intake of protein (Pitts and Lotspeich, 1946; Thompson and Barrett, 1954b).

C. Obligatory Cation-Anion Equivalence

The obligatory equivalence of total cation and total anion concentrations poses a relative restriction on the metabolism of any individual ion. Obviously in any electrolyte solution the sums of the free negative and positive charges are equal; furthermore, most ions in plasma and urine exist free in solution and are not associated with any particular species of oppositely charged ion.[†]

In the kidney, the reabsorbed fluid must contain equivalent amounts of cation and anion. A primary change in either net cation or net anion reabsorption must be accompanied by an equivalent change in the other. For example, when salts are given whose anions are poorly reabsorbed, cation excretion must increase. It should be noted that the excretion of equivalent quantities of oppositely charged ions, for example, sodium and sulfate, does not indicate that the clearances of the two ionic species are equal, since in this instance the concentrations in plasma differ markedly.

[†] Although the electrolytes of body fluids have generally been considered as completely dissociated into ions (except as modified by protein-binding or chelation), recent evidence indicates that interaction between oppositely charged ions in plasma and urine may have profound effects upon the metabolism of electrolytes. The infusion of sodium salts of multivalent anions leads to a marked increase in the excretion of potassium, calcium, magnesium, and strontium and an apparent reduction in the extent of ionization of extracellular calcium. Conversely, the infusion of magnesium chloride is often followed by a marked reduction in the tubular reabsorption of phosphate and sulfate. These findings have been interpreted as evidence for the existence of undissociated ion pairs in plasma and urine, formed by the association of a multivalent ion with an oppositely charged ion (Walser and Browder, 1958). The significance of this phenomenon in normal electrolyte metabolism remains to be determined.

D. Estimation of Tubular Transport

When more of a substance is filtered than is excreted it is often tacitly assumed that only reabsorption occurs. However, for potassium (see below), reabsorption may occur in one segment of the tubule, secretion in another. Thus, even though the direction of net movement is from plasma to tubular fluid in one portion of the tubule, the direction of net movement in the nephron as a whole may be in the opposite direction. It is possible that other minerals behave in a similar manner.

E. Mediation of Changes in Filtration Rate

The measured filtration rate represents the summated rates of filtration in each nephron. Since the mammalian kidney is composed of many nephrons (about one million in each kidney in man) whose individual filtration rates might vary considerably, the measured inulin or creatinine clearance provides only an indirect index of the filtration rate per nephron. A change in measured filtration rate might reflect (*1*) a greater change in filtration in some nephrons than in others, (*2*) a change in the number of functioning nephrons, or (*3*) a proportionate change in filtration in all nephrons.

When tubular function is evaluated in the presence of a varying filtration rate, it is of considerable significance to determine which of these factors accounts for the measured changes in filtration. A change in the number of functioning nephrons would not necessarily alter the tubular activity of the remaining nephrons. Consequently the calculated rate of tubular transport per unit volume of filtrate would remain constant. On the other hand, if a change in filtration rate were mediated by a proportionate change in filtration in all nephrons, then tubular transport might continue to function at a constant rate. However, if the transport process were operating at a rate dependent upon the availability of material within the lumen, the rate of transport would be altered.

Most studies dealing with the mechanism of the change in filtration rate have attempted to determine maximal rates of tubular transport, particularly of glucose. Potassium secretion has also been used as an index of tubular activity. Although its transport has not been characterized as having a maximal rate, it is believed to be independent of the composition and volume of the tubular fluid, provided that adequate amounts of sodium are available for exchange (see below). The following observations, although not entirely consistent, indicate that filtration rate (at least in the dog) changes principally as a result of alterations in filtration in *all* nephrons. Thus, the glucose T_m value is independent of filtration rate when the latter is varied by diet (Shannon *et al.*, 1941) or is reduced by lowering the arterial pressure within the aorta (Thompson and Barrett, 1954a). Potassium excretion remains constant during sodium loading when the filtration rate is reduced by clamping the renal artery (Davidson *et al.*, 1957). On the other hand, abdominal compression (Bradley and Bradley, 1947) or acute hypotension induced by drugs (Handley and Moyer, 1955) are reported to be associated with more or less proportionate reductions in the value of the glucose T_m.

F. Extrarenal Factors

In the interpretation of results of brief experiments on the intact animal, one cannot assume that renal function is influenced only by the observed changes in the electrolyte composition of the plasma and the measured changes in renal blood flow and filtration rate. Among the extrarenal factors which may influence acute experiments are secretion of hormones, the stimulation of renal nerves, and spontaneous diurnal variations. Until these factors become more accessible to measurement, the inference that their effects are negligible in brief experiments cannot be accepted without reservations.

G. Operational Concepts

Many of the parameters employed to describe renal function are operational concepts in the sense that they do not correspond to any physiochemical reality. This is notably true of certain imaginary fluids. Thus, the *reabsorbate* is the volume of fluid reabsorbed, and is calculated as the numerical difference between the filtrate and the excreted urine ; the term *tubular rejectate* has been introduced to denote the nature of the fluid that is not reabsorbed (hence rejected) following an acute procedure, such as administration of a diuretic ; the *free water clearance*, either positive or negative (see Section II), represents the difference between the observed urine volume and a volume of fluid containing the excreted solute at an osmolal concentration equal to that of plasma. By appropriate calculations, various solutes may be related to the volumes of the reabsorbate or of the rejectate, and hypothetical values may be derived for solute concentrations in these fluids. By definition, free water contains no solute. However, it is emphasized that these fluids do not exist as such within the kidney. For example, a given sodium concentration in the reabsorbate could be obtained by the reabsorption of sodium at one site in the tubule and by the reabsorption of water at another.

II. Water Excretion

The renal excretion of minerals necessarily involves the excretion of water. Under conditions when the total concentration of osmotically effective solutes in the urine is the same as in plasma, the excretion of water does not alter the effective osmotic concentration of the plasma and therefore does not change the distribution of water between cells and extracellular fluid. Effective osmotic concentration is defined as the total concentration of osmotically active solutes which do not freely traverse cell membranes. In both plasma and urine this is approximately proportional (under most circumstances) to the concentration of total cation and is independent of urea, a substance which freely enters cells. Broadly speaking, the excretion of urine at concentrations that are effectively isosmotic may be considered as influencing the volume but not the effective osmotic concentration of the extracellular fluid.

Water excretion by the kidney also serves a second function that is largely independent of the regulation of extracellular fluid volume, namely the regulation of the effective osmotic concentration of the extracellular fluid. This is achieved by variations in the concentration of osmotically effective solutes in the urine, chiefly through the action of the antidiuretic hormone (vasopressin). This substance is secreted by the supraopticohypothalamic system and is carried by the blood to the kidney where it acts to increase the solute concentration of the urine and. thereby, to lower the concentration of osmotically effective solutes in the body fluids (Verney, 1926). In the absence of vasopressin secretion, the concentration of osmotically effective solutes in the urine is lower than that of plasma, and consequently the effective osmotic concentration of the plasma tends to rise. This mechanism, together with the control of water intake by thirst, is responsible for the extremely narrow range of plasma osmolality in normal individuals.

The factors controlling the rate of vasopressin secretion are discussed in Vol. I, Chapter 12, and require only brief summary here. The principal stimulus to vasopressin secretion is the effective osmotic concentration of the extracellular fluid (Verney, 1947). Another stimulus appears to be a decrease in the volume of extracellular fluid or of plasma (Brun *et al.*, 1945). Additional stimuli are cold, pain, and certain emotions. Finally, a number of drugs, notably nicotine, are known to induce the secretion of vasopressin.

In addition to the secretion of vasopressin, its distribution and rate of destruction or excretion affect the amount of hormone reaching the kidney. Little is known at present about these factors (see Heller, 1957). However, it has been demonstrated that vasopressin disappears rapidly from the blood after injection and that it is inactivated principally by the liver and the kidney (Ginsburg and Heller, 1953).

A. The Mechanism of Urinary Dilution

In order to clarify the mechanism of action of vasopressin, it is helpful to consider first the renal reabsorption of water in the absence of the hormone, i.e., during water diuresis or in diabetes insipidus, when osmotically effective solutes are less concentrated in the urine than in the blood. Since the glomerular filtrate is virtually isosmotic with plasma, hypoosmotic urine can be achieved only by the reabsorption of solute without water or by the secretion of water without solute. In the absence of evidence for water secretion, it is presumed that urinary dilution is accomplished by the reabsorption of solute. Sodium salts are the only solutes which are present in the filtrate in amounts sufficiently large so that their removal can account for the observed rates of excretion of solute-free water. Thus the mechanism of dilution is thought to consist of the reabsorption of sodium salts.

The magnitude of free water excretion and the portions of the tubule involved in its formation are interrelated problems and may be considered together. Samples of fluid obtained from the midportions of the proximal tubule have been found to have an osmotic concentration indistinguishable from that of plasma during both antidiuresis and water diuresis (Walker *et al.*, 1941; Wirz, 1956). This has generally been interpreted as indicating that free water (either positive or negative) is formed distal to the proximal convoluted tubule. However, an almost unmeasurably slight degree of hypotonicity in the proximal tubule would also be consistent with the formation of large volumes of solute-free water in this segment (Mudge, 1954). For example, let us suppose that the urine flow is 9 ml/min, that the free water clearance is 6 ml/min, the filtration rate 100 ml/min and that solute and water are reabsorbed in constant proportions throughout the nephron. On the basis of these assumptions it can be calculated that halfway down the proximal tubule, where about 35% of the filtered solute has been reabsorbed, the osmotic concentration of the tubular fluid would be 97% of that of plasma. Clearly, analysis of samples taken from the distal end of the proximal tubule are required to exclude the participation of this segment in urinary dilution.

Nevertheless, it has generally been held that the proximal tubular fluid is isosmotic. Smith (1952) and his associates advanced the hypothesis that urinary dilution occurred within the distal tubule. It was inferred that this segment was impermeable to water. On the basis of early observations that free water clearance was constant during variable osmotic diuresis, it was conceived that the distal mechanism operates at a constant rate, provided that sufficient sodium is available in the lumen for reabsorption.

Subsequent experiments have focused attention on the relation between free water clearance and solute excretion. These observations have implications both with respect to the mechanism of free water formation and the location of this process within the nephron. In patients with nephrogenic diabetes insipidus, as well as in normal subjects during water loading, free water clearance at first rose rapidly as solute excretion increased, and then approached a limiting value (Orloff and Walser, 1956). An almost identical relationship between free water and solute excretion was obtained, whether solute excretion was increased by mannitol, acetazoleamide, or saline infusion. Mannitol and acetazoleamide decreased sodium reabsorption (possibly at different sites), whereas saline infusions increased the total amount of sodium reabsorbed. Thus, free water excretion was correlated with the amount of solute excreted rather than with the amount of solute (sodium salts) reabsorbed. If the diluting segment had a partial permeability to water, free water would diffuse out in response to the osmotic gradient at a rate which would increase as the fluid within the tubule became more dilute. Thus, the amount of free water excreted in the voided urine would be the result of two simultaneous opposing factors, one of which would depend on the *amount* of sodium reabsorbed and the other on the osmotic *concentration* of total solute remaining within the tubular fluid. This latter process is independent of the chemical nature of the residual solute. The data indicate that the latter effect is quantitatively at least as important as the former. Figure 1 shows how free water excretion might increase to approach a maximum under these conditions.

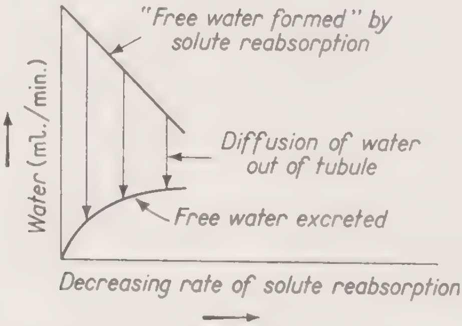
"Free water formed" by solute reabsorption
Diffusion of water out of tubule
Free water excreted
Water (ml./min.)
Decreasing rate of solute reabsorption

FIG. 1. Schematic representation of theoretical relationships between solute reabsorption and rates of water diffusion and free water excretion. For discussion see text.

It follows from this hypothesis that the amount of free water actually excreted must always represent only a portion of the volume of tubular fluid delivered to the diluting segment. In hydrated normal subjects, during solute loading, the free water excretion is about 15% of the glomerular filtration rate; therefore the inflow to the diluting segment must exceed this value. Its exact volume cannot be estimated at present. Participation of the proximal tubule in the diluting process cannot be excluded.

B. The Mechanism of Urinary Concentration

Since the urine formed under the influence of vasopressin is characteristically hyperosmotic to plasma, it has been presumed that this process involves the removal of water without accompanying solute from the tubular urine at some site in the nephron. The theoretical possibility remains that solute (without water) may be secreted into the tubular urine, but no evidence has been presented that substances known to be handled by tubular secretion contribute significantly to total urinary solute concentration. It was formerly thought that an active mechanism existed for the transport of water against an osmotic gradient (cf. Vol. I, Chapter 12). Inherent difficulties in this concept have been discussed by Brodsky *et al.* (1955).

Recent work by Koefoed-Johnsen and Ussing (1953), Andersen and Ussing (1957), and Sawyer (1957) has clearly demonstrated that posterior pituitary principles enhance water permeability of amphibian membranes. It is reasonable to suppose, therefore, that vasopressin influences the kidney by a similar mechanism of action.

The presence of hyperosmolality in the renal papilla of the rabbit was inferred by Ljundberg (1947) and has been amply confirmed in recent years in the rat (Wirz *et al.*, 1951), the hamster (Wirz, 1953), and the dog (Ullrich *et al.*, 1955 ; Berliner *et al.*, 1958). Smith (1951) speculated that this hyperosmotic area might be involved in the concentrating mechanism. The urinary dilution process may be counteracted by an increase in water permeability either at, or distal to, the sites of urinary dilution. The second process, i.e., concentrating the urine, requires the production and maintenance of an area of hyperosmolality in the renal parenchyma with which the tubular urine may equilibrate by diffusion. Increased water permeability in the hyperosmotic area would facilitate the formation of concentrated urine. This hypothesis has considerable appeal in that it permits vasopressin to overcome the effects of the diluting mechanism and also to concentrate the urine by a single action, i.e., the enhancement of the permeability of the tubule to water.

The micropuncture observations of Wirz (1956) have established that, in the rat, the contents of the first half of the distal convoluted tubule are hypoosmotic when the excreted urine is hyperosmotic, thus confirming and extending the earlier findings of Walker *et al.* (1941). These observations indicate that the tubular fluid does not become isosmotic until the second half of the distal tubule is reached. The second step, i.e., the formation of a hyperosmotic urine, occurs in a still more distal segment, i.e., the collecting ducts, as they traverse the papilla. This possibility had been inferred by Smith (1951) from the fact that animals which have long collecting ducts can form highly concentrated urine.

Hargitay and Kuhn (1951) and Wirz (1957) have suggested that the renal mechanism of concentrating the urine involves the countercurrent principle, both as a means of producing papillary hyperosmolality and as a means of preventing its dissipation.

The basic principle of the countercurrent process is illustrated by the mechanism which permits the feet of certain birds to be perfused with blood and yet maintain a temperature considerably below that of the rest of the body (Scholander, 1957). In these birds, the efferent vessels lie in close proximity to the venules that are returning the blood from the periphery. Therefore the arteries lose heat to the veins and the temperature of the blood falls progressively in the more distal portions of the limb. In this manner, the feet receive an adequate blood flow and their temperature remains close to that of the external environment without significant loss of body heat.

The manner in which the hyperosmotic region of the kidney is produced initially will be considered below. Its maintenance depends not only on the continued operation of the initial mechanism, but also on the countercurrent process which opposes those forces tending to dissipate the osmotic gradient. During hydropenia the osmolality of the renal papillae increases progressively from the outer medulla to the tips of the papillae (Ullrich *et al.*, 1955). This gradient is maintained by the free diffusion of water between incoming and outgoing blood vessels, in a manner analogous to the flow of heat in the example cited above. Thus an osmotic gradient in the direction of the long axis of the papilla can persist despite high rates of blood flow. According to this concept, at any level in the papilla the same osmotic concentration obtains for blood, interstitial fluid, and probably tubular cells. The osmolality within the tubular fluid will be considered below.

In addition to the flow of blood, a second force tending to dissipate the osmotic gradient is diffusion within the interstitial space in the direction of the long axis of the papilla. It may be noted that the length of the papilla in different species is correlated with the capacity to concentrate the urine. A long papilla tends to minimize the dissipation of the osmotic gradient by axial diffusion.

Wirz (1957), making use of a suggestion of Hargitay and Kuhn (1951), has proposed that the hyperosmotic area is produced by a combination of an active transport process and countercurrent exchange. Hargitay and Kuhn (1951) pointed out that a hairpin-shaped structure such as the loop of Henle can produce a considerable osmotic gradient in an axial direction if some force promotes the transfer of water from the incoming limb of the loop directly to the outgoing limb. Under these circumstances the contents of the loop become progressively concentrated until the bend is reached, and then are rediluted, finally reaching the original concentration (Fig. 2). According to Wirz, a cellular process might transport water from the incoming to the outgoing limb, or, alternatively, solute (sodium chloride) might be transported from the outgoing to the incoming limb. In addition, solute might be reabsorbed from the ascending limb and water diffuse passively out of the descending. Each process would require movement of large amounts of either water or solute across small concentration gradients. According to the first two mechanisms, in the steady state the amount of solute or water flowing into the loop would be equal to that flowing out at its distal end (except as modified by water or sodium reaching the loop from the collecting duct itself).

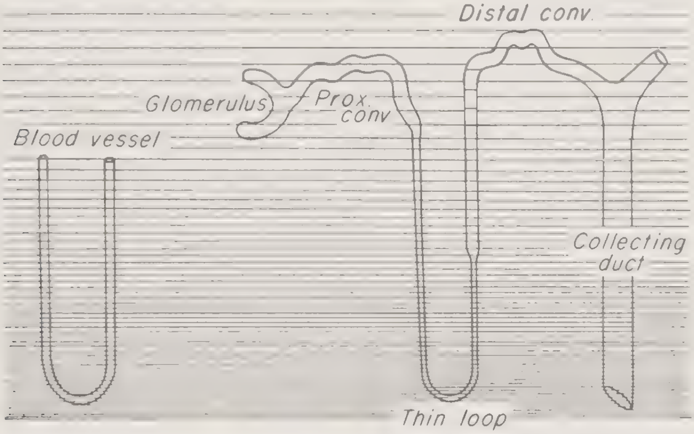

FIG. 2. Diagrammatic representation of osmolal concentration within the kidney according to theory of Wirz (1957). Osmolality is depicted as proportional to density of horizontal lines. Tubules within the cortex are isosmotic except for proximal portion of distal convoluted tubule which is hypoosmotic. Relationships are drawn for conditions under which urine is being maximally concentrated.

Berliner *et al.* (1958) have contended that the above mechanism would not function because it requires cellular permeability to water for the loop to influence the surrounding interstitial fluid, but simultaneously demands water impermeability for the contents of the loop to become concentrated. They have proposed a different process which does not involve a countercurrent principle for the thin loop itself. According to their concept the thin segment would actively transport sodium to the surrounding interstitial fluid, but would have a low permeability to water. The fluid within the loop therefore would become progressively diluted and the interstitial fluid would thus be hypoosmotic as it entered the distal convoluted tubule, where increased water permeability would allow approximate osmotic equilibration to occur. However, a recent report by Gottschalk and Mylle (1958) indicates that the contents of the loops of Henle are in fact isosmotic with those of the collecting ducts. Furthermore, the scheme of Wirz may be valid in view of the following theoretical considerations which are implicit in the concept of countercurrent. Let us suppose the tubule to be impermeable to water; then solute transported from ascending to descending limb would produce an osmotic gradient. This gradient would be displaced in an axial direction by the flow of fluid within the loop. If this flow were sufficiently rapid, the lateral gradient between adjacent points of the two limbs would become negligibly small. Now if we consider that the tubular epithelium is in fact permeable to water, there will be under these conditions no tendency for water to diffuse laterally from one limb to the other. Therefore, it follows that the establishment of an axial gradient is not dependent on the permeability characteristics of the membrane to water.

The manner by which this active countercurrent scheme might remove water from the surrounding tissue may be visualized as follows. In the process of establishing an axial gradient, osmotically active solute will have accumulated within the loop, and therefore the fluid leaving the loop during that time must have been relatively hypoosmotic. Continuation of this process could remove solute-free water diffusing into the loop from capillaries and interstitial fluid, thus making the entire papilla hypertonic. In a like manner, solute-free water could be removed from the collecting ducts.

The action of vasopressin is presently believed to result from an increase in the permeability to water of the proximal and distal convoluted tubules and of the collecting ducts. Presumably the active countercurrent process within the loop of Henle operates continuously, as indicated by the observation that hyperosmotic urine may be formed in the virtual absence of vasopressin under certain circumstances (see below). During water diuresis, the proximal tubule may deliver a larger volume of relatively hypoosmotic urine to the loop; during antidiuresis, a smaller volume of nearly isosmotic urine. Consequently, the maximal papillary osmolality that is attained during water diuresis is less than during antidiuresis (Ullrich *et al.*, 1955). As indicated above, in the presence of vasopressin the tubular fluid is hypoosmotic in the first half of the distal tubule and isosmotic in the second. According to the mechanism proposed by Wirz (1957) and Hargitay and Kuhn (1951) some degree of hypoosmolality of the fluid entering the distal tubule may be attributed to the active countercurrent process within the loop. The anatomical point at which this mechanism terminates is not known. However, in the area between the termination of the countercurrent process and the portions of the distal tubule accessible to puncture it is likely that sodium salts are actively reabsorbed. Furthermore, this area must have a limited permeability to water even under the influence of vasopressin. Ullrich (1958) has shown that sodium salts influence the oxygen consumption of tissue from the subcortical area of the medulla. This suggests an unusual property which may be related in some manner either to the process of sodium reabsorption or to an active countercurrent mechanism.

The detailed manner in which an active countercurrent mechanism might operate is subject to speculation. As Wirz pointed out, solute might be transported from ascending to descending limb, or water might move in the opposite direction. Whichever is the case, the interstitial fluid must be traversed in passing from one limb to the other. Therefore, it is likely that the active process is located in one limb only and that it is not a coupled mechanism involving both limbs. Furthermore, the anatomical difference between the thin descending limb and the thick portion of the ascending limb suggests a difference in function.

In the human kidney approximately one of every seven nephrons has a long loop of Henle which extends deep into the medulla (Smith, 1951). Consequently these nephrons may be chiefly responsible for the removal of solute-free water from the tubular fluid that is derived from all of the nephrons.

According to the countercurrent scheme, the site at which the urine becomes concentrated is in the collecting ducts. This has been considered the result of active cellular mechanisms of the epithelium of the loop of Henle rather than of the collecting duct itself. In a preliminary communication, Hilger *et al.* (1958) have implicated the collecting duct in the active transport of solute, indicating that the above concept may require modification.

C. The Relationship between Water and Solute Excretion

In order to express the magnitude of the renal regulation of osmotic concentrations, Ladd (1950) introduced the concepts of osmolal[†] clearance and free water clearance. Osmolal clearance, abbreviated C_{osm}, is a calculated rate of water excretion such that excreted solute, if dissolved in this volume, would form a solution isosmotic with plasma. Free water clearance refers to the difference between osmolal clearance and observed urine flow, and may be positive (abbreviated C_{H_2O}), negative (abbreviated $T^C{}_{H_2O}$) or zero, depending upon whether the urine is hypoosmotic, hyperosmotic, or isosmotic with the plasma, respectively. In algebraic terms, $C_{osm} = U_{osm}V/P_{osm}$, where U_{osm} and

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[†] Since freezing point depression is a function of osmolal concentration, rather than osmolar, the former term is used here (see Smith, 1956).

P_{osm} refer to urine and plasma osmolality, and V is urine flow: $T^{C}_{H_2O} = C_{osm} - V$ and $C_{H_2O} = V - C_{osm}$.

The principal advantage of describing water excretion in this way is that it circumvents the common tendency to evaluate renal concentrating or diluting function solely in terms of either the concentration of the urine or the rate of urine flow. Neither of these parameters alone is a reliable index of the extent of renal regulation. However, the free water clearance (either positive or negative) is an accurate measure of this process, since it takes into account both osmotic concentration and flow. When free water clearance is positive, its calculated value indicates the rate at which the kidney is removing solute-free water from the body and thereby tending to elevate the total osmotic concentration of the body fluids. The magnitude of negative free water clearance is an index of water conservation.

It has been noted previously that the total osmotic concentration of plasma (or urine) is not synonymous with the effective osmotic concentration. This difference is due largely to urea which is usually a small fraction of the total solute of plasma but is often a major portion of the total solute of urine. In order to measure the influence of renal function upon the effective osmotic concentration of the body fluids, it would be more appropriate to calculate free water clearance on the basis of the effective, rather than the total, osmotic concentration of the plasma and urine. For example, the excretion of hyperosmotic urine consisting chiefly of urea tends to *raise* the effective osmotic concentration of the plasma, because the amount of water excreted is proportionately greater than the amount of osmotically effective solutes. Thus, under these circumstances, even though the kidney is concentrating the urine, it is at the same time concentrating the body fluids.

The normal relationship between osmolal clearance and free water clearance at various rates of Pitressin administration is shown in Fig. 3. Observed urine flow is plotted against the osmolal clearance, and the free water clearance (positive or negative) is indicated by the vertical distance between the observed values and the diagonal line representing isosmotic urine ($U/P = 1$). This graph illustrates several important aspects of the relationship between excretion of solute and of water. During either water diuresis (labeled 0 ADH) or maximal antidiuresis, the concentration of the urine tends to approach that of plasma as solute excretion rises; nevertheless, the rate of free water excretion, positive or negative, also rises. Thus the magnitude of the renal concentrating or diluting process increases, even though the actual urine concentration is approaching an isosmotic value. The positive free water clearance rises continuously as solute excretion is increased, but at a diminished rate when flow is very high. During water diuresis

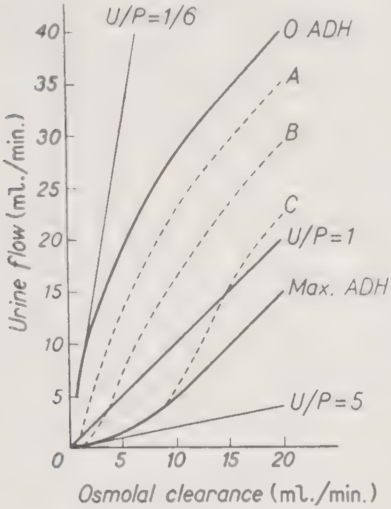

FIG. 3. Urine flow in man as function of solute excretion (osmolal clearance) during water diuresis and antidiuresis. Curves drawn by inspection from data recalculated to 70 kg body weight : water diuresis (Orloff and Walser, 1956 ; Walser and Orloff, 1957) ; maximal antidiuresis (McCance, 1945 ; Zak *et al.*, 1954) ; intermediate doses of Pitressin (Hollander *et al.*, 1957 ; Orloff *et al.*, 1958). The dashed lines A, B, and C represent in schematic fashion increasing rates of Pitressin administration. Free water clearance, positive or negative, is given by the vertical distance from a given point to the heavy diagonal line ($U/P = 1$), indicating isosmotic urine. The diagonal lines labeled $U/P = 1/6$ and $U/P = 5$ represent approximate limiting extremes of urine concentration in normal man. These limits are approached only at low rates of solute excretion, as indicated.

there is no well-defined point beyond which the free water clearance reaches a constant and maximal value, nor is there any minimal value at which the urine concentration becomes fixed when solute excretion decreases.

The situation during antidiuresis appears to be the opposite. At values of osmolal clearance over 5 ml/min, negative free water excretion tends to become constant. At low rates of solute excretion, the urine concentration reaches a maximal limiting value of about 1400 milliosmoles in man. At intermediate rates of administration of Pitressin the free water clearance increases towards a more positive value as solute excretion is increased ; i.e., levels of vasopressin which are sufficient to cause urinary hyperosmolality at low rates of solute excretion may be insufficient to do so at higher rates.

D. The Relationship between Filtration Rate and Water Excretion

The possible ways in which changes in filtration rate may be mediated have been discussed above. These same general considerations, applicable to substances having transport maxima, also apply to free water excretion when filtration rate changes. In a like manner, the interpretation of experimental data in regard to water excretion is obscured by uncertainty about the mechanism of the change in filtration rate.

These experimental difficulties may explain the conflicting results that have been obtained when the filtration rate is decreased during water diuresis. Shannon (1942) and others showed that dogs with experimental diabetes insipidus may elaborate a concentrated urine when severely dehydrated. del Greco and de Wardener (1956) and Berliner and Davidson (1957) found that acute reduction of renal arterial pressure may result in the excretion of hypertonic urine in the apparent absence of antidiuretic hormone. On the other hand, Chalmers *et al.* (1952), Leaf *et al.* (1954), and Bradley *et al.* (1955) found that somewhat similar procedures did not raise the concentration of the urine. Furthermore, patients with Pitressin-resistant diabetes insipidus did not excrete hypertonic urine when the filtration rate fell as a result of the administration of hypotensive agents or because of severe dehydration (Orloff and Walser, 1956). It is not possible at present to explain these conflicting results. The experimental study of this problem is hindered by the fact that even when the urine is highly dilute and of constant concentration, small amounts of antidiuretic hormone may still be secreted. This is indicated by the finding that ethanol, which is known to suppress the liberation of hormone, may produce a further decrease in urinary concentration during sustained water diuresis (Rosenbaum *et al.*, 1957).

During maximal antidiuresis, a reduction in filtration rate tends to decrease the concentration of the urine (Leaf *et al.*, 1954 ; Thompson and Barrett, 1954a). Systematic studies on this phenomenon have not been reported.

Although these observations indicate that the filtration rate may modify the concentration of the urine, there is abundant evidence that the role of glomerular filtration in regulating free water excretion is of minor importance in comparison with the role of antidiuretic hormone (Smith, 1951).

E. The Effect of Other Agents upon Free Water Excretion

The evidence cited above makes clear that changes in solute excretion, antidiuretic hormone secretion or destruction, and filtration rate can account for considerable variations in the flow and osmolality of the urine. Although a variety of other factors such as adrenal hormones, nervous stimuli, etc., have been studied with respect to their effect on water excretion, it seems probable that many of the reported results were mediated through one or more of the mechanisms discussed above.

On the other hand, free water excretion may be influenced by the specific nature of urinary solutes, independent of changes in total solute excretion, filtration rate, or antidiuretic hormone. This is particularly true of urea, which has less effect than other solutes upon urine flow whether given during water diuresis (Ludemann *et al.*, 1951 ; Rosenbaum *et al.*, 1953) or antidiuresis (Ambard and Papin, 1909 ; Rubini *et al.*, 1957 ; Epstein *et al.*, 1957).

III. Sodium and Chloride

The renal regulation of sodium excretion is accomplished by the balance between glomerular filtration and tubular reabsorption. For operational purposes, reabsorption may be divided into three categories : first, sodium reabsorption with fixed anions (mostly chloride) ; second, sodium reabsorption associated with reabsorption of bicarbonate and with net acidification ; and thirdly, the reabsorption of sodium in exchange for potassium. Under normal conditions, about 78 % of the filtered sodium is reabsorbed as part of the first process, 18 % with the second, and 3 % with the third. The mechanisms for urinary acidification and for potassium excretion are discussed in subsequent sections of this chapter.

As far as is known, the chloride ion undergoes no specific exchange reactions with other anions from the tubular cells. Its removal from the tubular fluid occurs almost exclusively in association with sodium reabsorption and to only a minor extent in association with the movement of other cations. Consequently, the reabsorption of chloride may be considered together with that of sodium. Since sodium and chloride ions constitute the major portion of the osmotically effective solutes of the extracellular fluid, their rate of excretion is intimately associated with osmoregulation (see Section II).

A. Cellular Mechanisms

The cellular mechanisms by which sodium and chloride are removed from the tubular fluid are not known. It has generally been held that isosmotic reabsorption is accomplished by the active removal of sodium and chloride resulting in the passive diffusion of water out of the tubule (Wesson and Anslow, 1948 ; Mudge *et al.*, 1949). This concept is based largely on the observation that osmotic diuresis evokes a relatively greater excretion of water than of sodium and chloride. Under these conditions, urine is nearly isosmotic with plasma. The derived data indicate that sodium and chloride are reabsorbed against a large concentration gradient. It has been inferred, therefore, that this is accomplished by an active cellular process. The conclusion that sodium and chloride are actively reabsorbed and that water diffuses out of the tubular lumen is also supported by the findings obtained during the process of urinary dilution (see Section II).

The lumen of the tubule is electronegative with respect to the peritubular fluid (Solomon, 1956; Giebisch, quoted by Pitts, 1958). This has been taken to indicate that the movement of chloride is a passive consequence of the electrochemical gradient established by the active removal of sodium. The alternative possibility, that chloride is transported actively, has been proposed on the basis of the action of mercurial diuretics, agents which increase the excretion of chloride relatively more than that of sodium. However, this may not necessarily indicate that the primary action of mercurials is on chloride transport. The primary inhibition of proximal sodium reabsorption would require that an equivalent amount of anion remain within the tubular fluid. If at a more distal site sodium were to be reabsorbed by exchange for either hydrogen or potassium, and if no chloride were removed in this segment, then the voided urine would contain relatively more chloride than sodium, even though the primary action of the diuretic had been on sodium reabsorption (Berliner, 1958). In addition, it has been observed that the plasma level of chloride is more critical than that of sodium in determining the magnitude of mercurial diuresis (Axelrod and Pitts, 1952).

Attempts to study the basic mechanism of sodium and chloride reabsorption with metabolic inhibitors have not yielded conclusive results either with respect to the mechanism or its energy requirements.

B. Factors Influencing Sodium and Chloride Excretion and Reabsorption

Under normal circumstances the amounts of sodium and chloride that are excreted in the urine are only a small fraction (about one per cent) of the amounts filtered. It is therefore difficult to measure the magnitude of the reabsorptive process and to evaluate the manner in which changes in reabsorptive rate determine sodium excretion. Hypothetical data are presented in Table I which illustrate the changes that can be

TABLE I

METHODS OF CALCULATING SODIUM REABSORPTION[a]

Original data					Derived data				
Experimental conditions	$U_{Na}V$ µeq/min	V ml/min	P_{Na} µeq/ml	GFR ml/min	$P_{Na}GFR$ µeq/min	T_{Na} µeq/min	$\frac{T_{Na}}{GFR}$ µeq/ml	$\frac{T_{Na}}{(GFR-V)}$ µeq/ml	$\frac{T_{Na}}{P_{Na}GFR}$ %
Control	100	1	140	100	14,000	13,900	139	140	99
Isotonic saline	1000	7	140	120	16,800	15,800	132	140	94
Hypertonic saline	1000	4	149	110	16,400	15,400	140	145	94
Mannitol	1000	14	130	100	13,000	12,000	120	140	92

[a] Hypothetical data depicting sodium excretion under one control and three experimental conditions. Table constructed to make rates of excretion equal during experimental periods. Values are representativ reported observations. For explanation of symbols, see text.

obtained when sodium excretion is increased by three different experimental procedures, i.e., the administration of isotonic saline, hypertonic saline, or mannitol. In each instance, the rate of sodium excretion is assumed to increase by the same amount. However, as indicated by the derived data, the changes in reabsorption vary, depending upon the parameter chosen for consideration. The amount of sodium reabsorbed per unit time (T_{Na}) increases in both types of saline diuresis. This is associated with an increase in the amount filtered ($P_{Na} \times GFR$) that is approximately, but not exactly, proportional to the change in the amount reabsorbed. During mannitol diuresis, T_{Na} decreases. In this particular set of figures, this is in part dependent on a decrease in the amount of sodium filtered ; however, the same phenomenon is observed when the filtered sodium remains constant or is increased (Mudge *et al.*, 1949). The amount of sodium reabsorbed per volume of glomerular filtrate (T_{Na}/GFR) decreases with isotonic saline and with mannitol, but increases with hypertonic saline. When considered in terms of the volume of water reabsorbed ($GFR - V$), sodium reabsorption remains constant in two instances, but is significantly elevated during hypertonic saline diuresis. The per cent filtered sodium that is reabsorbed is given in the final column. Although this value is decreased in each of the experimental situations, the magnitude of the change is not constant. Thus, although urinary excretion increases by the same amount, reabsorption decreases, increases, or remains constant, depending upon the method of evaluation.

C. The Nature of Osmotic Diuresis

The rapid infusion of poorly reabsorbed solutes, such as urea or mannitol, is accompanied by a rise in urine flow and an increase in the rate of sodium excretion. This increase is associated with decreased reabsorption of sodium (T_{Na}) and has been attributed to changes in the concentration of sodium within the tubular fluid (Wesson and Anslow, 1948 ; Mudge *et al.*, 1949). It is believed that the major portion of the glomerular filtrate is reabsorbed by proximal reabsorption and that the tubular fluid in this segment remains isosmotic or nearly so (see Fig. 4). Under normal circumstances, sodium and chloride are the major solutes of the filtrate. Hence, the removal of sodium and water in isosmotic proportions has relatively little effect upon the composition of the fluid remaining within the tubule. Normally, the concentration of endogenous urea progressively rises in the tubular fluid but remains low, relative to sodium, until most of the glomerular filtrate has been reabsorbed. During osmotic diuresis, the relative amounts of sodium and unreabsorbed nonelectrolyte in the tubular fluid are altered. The reabsorption of sodium and chloride is still accompanied by the removal of water in isosmotic or nearly isosmotic proportions. However, the nonelectrolytes now make a far greater contribution to the total osmotic concentration. Consequently a decrease in the sodium concentration of the tubular fluid occurs early in the reabsorptive process. As the fluid progresses down the tubule, sodium reabsorption thus occurs from a fluid in which the sodium concentration is progressively decreasing. Yet sodium excretion increases. This raises the possibility that at every point in the tubule the amount of sodium reabsorbed is influenced by the local concentration of sodium in the tubular fluid. For the entire segment, the total amount reabsorbed (T_{Na}) would be approximately proportional to the areas under the curves given in Fig. 4. A mathematical formulation derived with these assumptions has been shown to predict the rate of sodium excretion with moderate precision during mannitol diuresis (Walser, 1956).

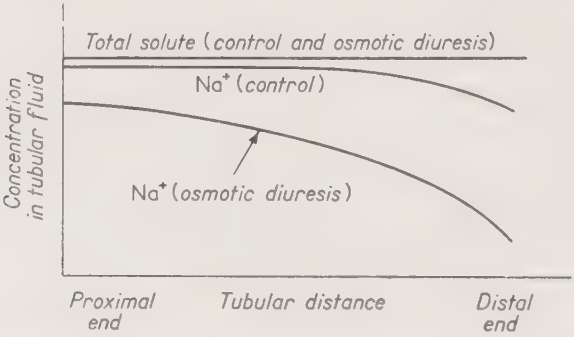

FIG. 4. Schematic diagram of sodium concentration in the tubular fluid of the proximal isosmotic segment. Normal compared to osmotic (nonelectrolyte) diuresis; total solute concentration in glomerular filtrate assumed identical in both conditions.

D. Effect of Filtration Rate

In brief experiments, a fall in the rate of glomerular filtration is generally associated with a decrease in the amount of sodium and chloride excreted. Attempts to describe this effect in quantitative terms have been reviewed by Wesson (1957). Factors which may be involved include a reduction in the amount of sodium filtered and an increase in the relative contributions of the proximal as opposed to the distal process of sodium reabsorption. An alteration in intraluminal pressure is also likely to take place and may have an effect on the reabsorptive mechanism (Gottschalk and Mylle, 1956).

In prolonged experiments, comparable changes in filtration rate are not associated with a uniform effect on sodium excretion. Due to probable secondary changes in extracellular fluid volume, adrenal cortical activity, or autonomic function, it has not been possible to evaluate intrinsic renal mechanisms in experiments of this type (see Mueller *et al.*, 1951).

When fluctuations in filtration rate in either brief or prolonged experiments are within the error of measurement, it is not possible to determine whether an accompanying change in sodium excretion is attributable to glomerular or tubular factors. Obviously, if a significant fall in filtration rate is associated with an increase in sodium excretion, this increase may be ascribed to an effect upon tubular activity. Such a response is elicited by a number of diuretic drugs and indicates that they act on the tubule.

E. Adrenocortical Steroids

The adrenal steroids have two actions on the kidney which affect the rate of sodium excretion in opposite ways, i.e., they increase the rate of glomerular filtration and they promote the tubular reabsorption of sodium and chloride. Furthermore, administration of adrenal steroids tends to increase extracellular fluid volume which may in itself increase filtration rate. Conversely, adrenalectomy leads to a loss of extracellular fluid volume and a fall in filtration rate. It is not surprising, therefore, that the measurement of sodium excretion alone leads to conflicting conclusions as to actions of these hormones upon the kidney.

The measurement of extracellular fluid volume, difficult at best, becomes further complicated by the fact that adrenal steroids may alter measured " spaces " independent of changes in sodium balance (Sweet *et al.*, 1958 ; Walser *et al.*, 1955).

In adrenal insufficiency the filtration rate may not be appreciably reduced if adequate salt is given (Wirz, 1945 ; Lotspeich, 1949 ; Roemmelt *et al.*, 1949). However administration of aldosterone (but not of desoxycortisosterone) leads to an elevation of filtration rate independent of sodium retention (Garrod *et al.*, 1955 ; Liddle *et al.*, 1955). In normal dogs similar changes in filtration rate are observed following steroid administration (Davis and Howell, 1953), but it has not been shown that filtration rate in normal humans can be significantly increased by corticosteroids unless there is simultaneous retention of sodium. Accordingly adrenal steroids produce retention of administered sodium in normal or hypoadrenal humans, but have a variable effect on sodium excretion in normal dogs.

The effects of aldosterone and other steroids on sodium excretion may be considerably modified by changes in the effective osmolality of the plasma (Bartter *et al.*, 1956). These interrelationships are considered in detail in Vol. I, Chapter 12 and Vol. II, Chapter 25.

F. Vasopressin (cf. Vol. I, Chapter 12.)

The antidiuretic hormone vasopressin has long been known to promote the excretion of sodium and chloride under certain conditions. This effect is variable and somewhat controversial results have been obtained. Normal animals are almost continuously secreting vasopressin (as evidenced by the fact that normal urine is usually hyperosmotic); consequently it is not surprising that neither Pitressin nor purified vasopressin has reproducible effects upon electrolyte excretion when given without pretreatment. When administered following hydration, both Pitressin and purified vasopressin augment sodium and chloride excretion (Anslow and Wesson, 1955; Brooks and Pickford, 1958). This response is exaggerated if overhydration develops as a result of the ensuing antidiuresis; in this instance, aldosterone secretion may be inhibited by expansion of extracellular fluid volume (Bartter *et al.*, 1956).

G. Adrenal Medullary Hormones and Renal Nerves

The autonomic control of the renal circulation is indirectly concerned with the excretion of electrolytes, principally by regulation of glomerular filtration rate. Humoral, nervous, and intrinsic renal mechanisms are integrated in the regulation of renal hemodynamics in a manner as yet incompletely understood. Accordingly the role of epinephrine, norepinephrine, and nervous stimuli in regulating sodium excretion remains to be clarified. This subject has recently been reviewed in detail by Bradley (1957).

IV. Potassium

Analysis of the renal regulation of potassium excretion is complicated by the observation that potassium is both reabsorbed and secreted by the renal tubules. That potassium is reabsorbed can be inferred from the fact that most mammals normally excrete an amount equivalent to about 10% of the amount filtered. Tubular secretion, as indicated by a potassium clearance greater than the filtration rate, is seen under certain experimental and pathological conditions. These observations raise the possibility that reabsorption may be occurring in one segment of the tubule at the same time that secretion occurs in another. This might be the case whether the potassium clearance is less than or greater than the filtration rate.

In the intact animal it is impossible to quantify the exact contributions of the reabsorptive and secretory processes, but indirect evidence indicates that changes in the rate of tubular secretion, rather than of reabsorption, are chiefly responsible for variation in the rate of potassium excretion.

This evidence can be summarized as follows. (1) Increased potassium excretion is associated with increased intake (Berliner *et al.*, 1950).

hyperosmolality (Mudge *et al.*, 1950b), acute alkalosis (Franglen *et al.*, 1953), and the administration of carbonic anhydrase inhibitors (Berliner *et al.*, 1951). These increments are probably attributable to increased secretion, rather than decreased reabsorption, since in each instance the amount of potassium excreted may exceed that which is filtered. (2) Potassium excretion remains constant with acute reduction in the filtration rate of moderate degree, provided that sodium is present in the urine (Davidson *et al.*, 1957). The rate of excretion of other electrolytes falls under similar circumstances. The failure of potassium excretion to change is most simply attributed to a constant rate of secretion associated with virtually complete reabsorption of the variable amounts filtered. (3) Many of the relationships between potassium excretion and acid-base balance are also interpreted most simply on the basis of the secretory mechanism (see below). Although the concept of complete reabsorption is supported by the above findings, the possibility remains that it may be incomplete, particularly during diuresis brought about by the administration of unreabsorbed solutes such as mannitol or sodium sulfate.

The localization of potassium reabsorption and secretion within the nephron has been studied by several techniques. Bott (1954) showed that samples of proximal fluid obtained by micropuncture contained less potassium than did distal fluid and that in the distal samples the concentration of inulin rose less than that of potassium. It was concluded, therefore, that potassium was added to the urine in the distal tubule. The recent application of stop-flow analysis by the technique of Malvin *et al.* (1957) has contributed additional evidence for distal secretion (see Pitts, 1958, see also Vol. II, Chapter 26). Furthermore, studies with radioactive isotopes suggest that the distal tubule is permeable to potassium (Black and Emery, 1957). In a preliminary report Hilger *et al.* (1958) have obtained samples of fluid from the collecting duct which indicates that potassium may be secreted by this structure.

The nature of the reabsorptive mechanism is not known. One proposal is that the reabsorption of both sodium and potassium is secondary to that of chloride and that the cations are reabsorbed in the same proportions in which they are present in the glomerular filtrate (Koch *et al.*, 1956). This hypothesis is based on experiments in which the urinary excretion of sodium and potassium was varied over a wide range. However, it cannot be accepted without reservation since other evidence (see below) indicates that the secretion of potassium may be influenced by the sodium content of the tubular fluid.

Potassium secretion involves exchanges with sodium rather than secretion with an anion. This was demonstrated by Berliner *et al.* (1950) who infused salts of the nonreabsorbable anions, thiosulfate and ferrocyanide. The amount of potassium added to the urine by tubular secretion exceeded the amount of chloride, phosphate, and sulfate excreted. Therefore, potassium was added to the tubular fluid by exchange for a cation, namely sodium.

Factors which influence the sodium-potassium exchange mechanism may be subdivided into those which pertain to the composition of the tubular fluid at the site of exchange, and those which pertain to cellular factors. The latter include (*a*) intracellular potassium concentration, (*b*) intracellular hydrogen ion concentration, and (*c*) mineral-corticoid activity.

(*a*) The cellular potassium concentration of the kidney is not subject to direct measurement in the intact animal. Studies by indirect means indicate that potassium secretion is responsive to intracellular potassium concentration (Mudge *et al.*, 1950a ; Berliner *et al.*, 1950).

(*b*) Intracellular hydrogen ion concentration is intimately related to potasssium metabolism. This is true of many other tissues besides the kidney. The renal mechanisms have been most intensively studied in acute experiments which have demonstrated an inverse relationship between the excretion of potassium and hydrogen ion (Berliner *et al.*, 1951). Thus, the infusion of neutral potassium salts increases bicarbonate excretion ; either metabolic or respiratory alkalosis increases potassium excretion (at least transiently) in conjunction with alkalinization of the urine (see Orloff, 1956) ; metabolic acidosis decreases potassium output when other physiological variables apparently remain constant (Berliner *et al.*, 1954) ; and chronic potassium depletion may be accompanied by acid urine even in the presence of extracellular alkalosis. Berliner *et al.* (1950) have proposed that sodium-potassium exchange in the distal tubule competes with sodium-hydrogen exchange so that potassium and hydrogen secretion vary inversely. They have postulated that the same carrier mechanism is involved in both exchange reactions. Tissue analyses of kidney cortex suggest that this inverse relationship may depend directly on the relative intracellular concentrations of potassium and hydrogen ion (Anderson and Mudge, 1955).

(*c*) The adrenal steroids stimulate the reabsorption of sodium and chloride and are believed to enhance the tubular secretion of potassium in exchange for sodium. Potassium excretion is reduced after adrenalectomy and is increased by adrenal steroids. Although these effects could conceivably reflect alterations in the reabsorption of potassium, the manner in which they are modified by sodium metabolism (see below) supports the interpretation that they are mediated through changes in secretion. Although adrenal steroids apparently increase sodium-potassium exchange, they are not essential to it, since the adrenalectomized dog is capable of secreting potassium (Brazeau, 1952).

The composition of the tubular fluid at the site of sodium-potassium exchange influences the rate of exchange mainly by virtue of its sodium concentration. Acute increases in sodium excretion are generally accompanied by increased potassium excretion. A reduction in sodium excretion produced by lowering the filtration rate is accompanied by a fall in potassium excretion (Bradley *et al.*, 1955). However, if the urinary excretion of sodium is maintained at a high level, potassium excretion remains constant as the filtration rate falls (Davidson *et al.*, 1957). In a subject receiving adrenal steroids, increased potassium excretion is observed only if sodium is provided in the diet. Under these circumstances sodium presumably becomes available for exchange with potassium in the tubule (Seldin *et al.*, 1956).

Certain drugs have specific effects on potassium excretion. Acetazolamide and other inhibitors of carbonic anhydrase lead to a pronounced rise in potassium excretion in association with alkalinization of the urine. This effect is chiefly due to stimulation of potassium secretion (Berliner *et al.*, 1950). Mercurial diuretics have a variable effect on potassium excretion. When potassium output is initially low, a slight increase may result. When potassium output is initially high, mercurials depress its excretion (Mudge *et al.*, 1950a). This latter effect has been attributed to an inhibition of the secretory mechanism and has been directly demonstrated in the chicken in which a renal portal circulation is present (Orloff and Davidson, 1956).

V. Acidification of the Urine

A. Introduction

The kidneys bear the major responsibility for the regulation of both the total quantity and relative proportions of fixed cation and fixed anion in the body fluids. For purpose of describing renal function, fixed ions may be considered as those which cannot be metabolized and which do not have buffer capacity, in contrast to labile ions which may be synthesized or degraded by metabolic reactions or which do have buffer properties within the physiological range of pH. In the glomerular filtrate, bicarbonate is the predominant labile ion ; in the excreted urine, ammonium, phosphate, creatinine, and small amounts of organic cations and anions constitute the major labile ions. The quantity of labile cations in the urine may thus be either greater or less than the quantity of labile anions. The labile ions in plasma, on the other hand, are all anions. In other phases of the body fluids, the total concentration of labile anions differs from that of plasma. Although the kidney operates on an ultrafiltrate of plasma, its contribution to total acid-base balance is not confined to the plasma but is reflected throughout the body fluids. Thus, the kidney effectively regulates the total labile anion content.

Although bicarbonate constitutes most of the labile anion of the plasma, extracellular bicarbonate represents less than half of the total body buffers (Swan and Pitts, 1955). The fraction of the total labile anion consisting of bicarbonate is not regulated by the kidney but by the lungs. The respiratory center controls alveolar carbon dioxide tension which in turn determines the carbon dioxide tension of arterial blood and of the tissues. The pH of each phase of the body fluids may be expressed in terms of the ratio of bicarbonate to dissolved carbon dioxide, in accordance with the Henderson-Hasselbalch equation†; the higher the ratio, the higher the pH. This equation also applies to all other buffer pairs such as the nonbicarbonate buffers of plasma. It follows from this relationship that, in response to changes in carbon dioxide tension, plasma will exhibit a change in the cationic equivalence of nonbicarbonate buffers as compared to bicarbonate. A rise in carbon dioxide tension ($p\mathrm{CO_2}$) will effect an increase in bicarbonate concentration and a decrease in the cationic equivalence of nonbicarbonate buffers without any change in total labile anion. In this manner the kidneys and the lungs share the control of extracellular pH, the former regulating total labile anion content and the latter the partial pressure of $\mathrm{CO_2}$.

Protein catabolism in man normally produces an excess of fixed anions over fixed cations of about 75 meq per day, which is excreted by the kidney. It is worthy of note, however, that the major acid product of metabolism, carbon dioxide, is excreted by the lungs.

B. Means of Describing Acidification of the Urine

There are several ways in which experimental data may be used to describe the acidifying process. A representative value according to each method is given in Table II.

1. In terms of homeostasis, the most pertinent parameter is the rate of "net acidification." This is measured as the sum of titratable acid plus ammonium excretion, minus bicarbonate excretion: it may be positive or negative.

2. The pH of the urine is also of significance, particularly in relation to those aspects of acidification which involve the hydrogen ion gradient between plasma and tubular fluid. However, the pH may bear little relationship to net acidification, as it gives no indication of the amount of ammonium or buffers present.

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† $\mathrm{pH} = \mathrm{p}K' + \log([A^-]/[\mathrm{H}A])$, where $\mathrm{p}K'$ is the negative logarithm of the apparent dissociation constant of the buffer pair, and $[A^-]$ and $[\mathrm{H}A]$ are the concentrations of the proton acceptor and donor, respectively.

TABLE II

PARAMETERS OF ACIDIFICATION†

Observed Data		Methods of Expressing Acidification	
$P_{HCO_3^-}$	25 meq/liter	1. " Net acidification "	51 μeq/min
GFR	110 ml/min	2. Urine pH	6.4
V	10 ml/min	3. Bicarbonate reabsorption	
$U_{HCO_3^-}V$	24 μeq/min	*a.* $T_{HCO_3^-}$	2726 μeq/min
$U_{TA}V$	50 μeq/min	*b.* $T_{HCO_3^-}/(GFR - V)$	27.3 meq/liter
$U_{NH_4^+}V$	25 μeq/min	*c.* $T_{HCO_3^-}/GFR$	24.7 meq/liter
Urine pH	6.4	*d.* $[U_{HCO_3^-}V/(P_{HCO_3^-} \times GFR)]$	0.88%
		4. " Total hydrogen ion secretion "	2801 μeq/min

† Observed data are representative values for normal man.

3. Bicarbonate reabsorption has been widely utilized in recent years as an index of the magnitude of the acidification process. The problems in expressing bicarbonate reabsorption in a meaningful way are similar to those involved in expressing sodium reabsorption. Parameters that have been used are the amount reabsorbed per unit time $T_{HCO_3^-}$, the ratio of bicarbonate reabsorbed to water reabsorbed $T_{HCO_3^-}/(GFR - V)$, the bicarbonate reabsorption per unit volume of glomerular filtrate $(T_{HCO_3^-}/GFR)$, or the fraction of filtered bicarbonate excreted $U_{HCO_3^-} \times V/P_{HCO_3^-} \times GFR$. Each calculation yields a different value and may give rise to different interpretations.†

4. " Total hydrogen ion secretion " by the tubules has been calculated as the total amount of acid that would theoretically have to be added to the glomerular filtrate to account for the bicarbonate reabsorbed plus the titratable acid and ammonium excreted.

C. Tubular Localization of Processes of Acidification

Only a few direct observations of the pH and bicarbonate content of tubular fluid have been reported. Montgomery and Pierce (1937), working with amphibian kidneys, found that the urine does not become acid until it reaches the distal segment. Chloride concentration was reported by Walker *et al.* (1941) to rise in the proximal segment in mammals, implying that bicarbonate concentration fell. Using the stop-flow technic, Malvin *et al.* (1958) have obtained indirect evidence

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† It may be pointed out that the kidney tends to adjust extracellular bicarbonate concentration towards the value $T_{HCO_3^-}/(GFR - V)$, rather than $T_{HCO_3^-}/GFR$. At high rates of urine flow these values may differ significantly. Since a volume equal to approximately ten times the volume of extracellular fluid is reabsorbed by the kidney each day, it follows that the concentration of bicarbonate in extracellular fluid cannot differ very much from its concentration in the reabsorbate $(GFR - V)$ except during experiments with abnormal electrolyte loads.

for the same conclusion. However, Giebisch (1956) found no change in chloride concentration or in the pH of samples obtained from the proximal segment of *Necturus*. This finding is consistent with the results obtained in the dog by Pitts (1958) with the stop-flow technic. The conflicting results obtained with stop-flow remain to be resolved. The micropuncture observations suggest a species difference. It is also possible that the pH of the samples of tubular fluid changed after collection and before measurement (see below). The resolution of this problem is fundamental to an understanding of bicarbonate reabsorption.

D. The Cellular Mechanisms of Urinary Acidification

The renal excretion of acid entails three processes : (1) the reabsorption of filtered bicarbonate ; (2) the substitution of hydrogen ions (in the form of titratable acid) for fixed cation ; and (3) the substitution of ammonium ions for fixed cation. The question is whether these processes occur throughout the nephron as a result of a single cellular mechanism, or whether they involve cellular operations that are fundamentally different. If the pH falls from the glomerulus to the end of the nephron [as inferred by the findings of Walker *et al.* (1941)], titratable acid will rise progressively. On the other hand if the findings of Giebisch (1956) are applicable to mammals, it follows that the major portion of bicarbonate reabsorption occurs in the presence of a constant concentration of bicarbonate in the tubular fluid, and that water, sodium, chloride, and bicarbonate are all removed in the proportions present in the filtrate. For the purposes of the present discussion it is assumed that the latter is correct.† Thus, acidification is assumed to involve two steps : first, bicarbonate reabsorption at constant concentration ; and second, at a more distal site, net acidification resulting in a fall in the bicarbonate concentration of the tubular fluid.

1. *Bicarbonate Reabsorption at Constant Concentration*

The role of carbonic anhydrase in this process is of major significance. Early studies with relatively weak enzyme inhibitors, such as sulfanilamide, were interpreted as indicating that proximal reabsorption is independent of carbonic anhydrase activity (Pitts and Alexander, 1945). However, following the administration of the much more potent inhibitor, acetazolamide, a major portion of the filtered bicarbonate may be excreted, indicating that the enzyme is involved in proximal reabsorption (Berliner *et al.*, 1951). Although the kidney contains a large amount of carbonic anhydrase, it is not clear whether the enzyme is present within the tubular fluid. Carbonic anhydrase infused intravenously appears in the urine (Ochwadt and Pitts, 1956), but normally none can be detected by available methods. In the evaluation o mechanisms of bicarbonate reabsorption it is of central significance whether the enzyme normally acts within the tubular fluid or only within the cells.

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† It has not been determined to what extent the changes in osmolality of the tubular fluid along the course of the nephron are reflected by changes in its bicarbonate concentration. During antidiuresis this may be of special importance in the thin loop and in the early portion of the distal convoluted tubule, where the fluid may be concentrated and diluted, respectively.

In normal man, approximately 3 millimoles of bicarbonate per minute are filtered, the proximal tubule reabsorbing most of this. One way by which this might be accomplished is by adding hydrogen ions to the tubular fluid, converting bicarbonate to carbonic acid. The decomposition of carbonic acid to carbon dioxide and water would result in the diffusion of carbon dioxide out of the tubule. The rate of the dehydration reaction would therefore approach 3 millimoles/min. Figure 5 illustrates the rate of the uncatalyzed reaction at constant bicarbonate and carbon dioxide concentrations as calculated from the equation of Roughton (1944), assuming that 40 ml of proximal tubular fluid are involved. It is seen that the pH of the entire proximal tubular fluid would have to be maintained at approximately 6.4 for reabsorption of the filtered bicarbonate to occur by this means, unless carbonic anhydrase were present in the lumen.

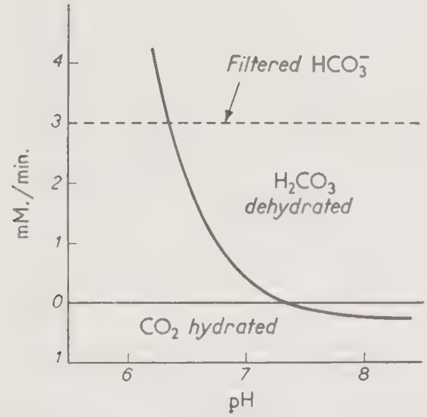

FIG. 5. Uncatalyzed rate of hydration—dehydration of carbonic acid at 37°C, for 40 ml of fluid containing constant bicarbonate concentration of 24 m moles/liter and a constant partial pressure of carbon dioxide of 40 mm Hg. Catalysis by inorganic constituents is considered negligible. See Roughton and Clark (1951).

The pH of the tubular fluid under conditions of *disequilibrium* is not given by the conventional form of the Henderson-Hasselbalch equation, $pH = 6.1 + \log([HCO_3^-]/[CO_2])$, but must be calculated from the carbonic acid concentration, i.e. $pH = 3.2 + \log([HCO_3^-]/[H_2CO_3])$.† If the hydration reaction were permitted to reach equilibrium, the pH of the tubular fluid (Fig. 5) would rise from 6.4 to nearly 7.4. The findings of Giebisch (1956) are thus consistent with fairly acid proximal fluid *in vivo.* If, on the other hand, carbonic anhydrase were available to the fluid within the lumen of the tubule, the pH would remain at approximately 7.4, even though hydrogen ions were being added at the same rate as calculated for the uncatalyzed reaction.

An alternative possibility, considered below, is that bicarbonate ions as such, rather than as CO_2, are removed from the proximal tubular fluid, along with sodium, chloride, and water. Under this circumstance, a constant pH could be maintained without the need for intraluminal carbonic anhydrase.

Whatever the specific cellular mechanism which initiates proximal bicarbonate reabsorption, carbonic anhydrase is likely to be involved within the renal tubular cells. Whether bicarbonate or hydrogen ions are transported, they probably proceed through areas in which the pH is different from that of plasma. Consequently at each cell interface there may occur a partial interconversion of bicarbonate and carbonic acid. This would involve the hydration-dehydration reaction (if it is assumed that the concentration of dissolved carbon dioxide remains constant). Furthermore, carbonic anhydrase may facilitate the net movement of bicarbonate ions within the cell or in the interstitial fluid by permitting part of this movement to occur as the diffusion of carbon dioxide in the direction of bicarbonate transport and the diffusion of hydrogen ions in the opposite direction.

Thus, whether or not carbonic anhydrase is present in the lumen and whether bicarbonate or CO_2 is removed from the tubular fluid, the enzyme is likely to enhance bicarbonate reabsorption in the proximal portion of the tubule. Consequently, the experimental observations made with acetazolamide cannot be interpreted to lend support to one mechanism in preference to another.

Indirect evidence favoring one or the other process may however be obtained from considerations of the electrochemical gradients involved. The bicarbonate concentration gradient from proximal tubular urine to plasma is very small (Giebisch, 1956). Furthermore, the lumen is −20 mv with respect to plasma (Solomon, 1956). Consequently, the electrochemical gradient is in a direction to favor bicarbonate ion reabsorption. The hydrogen ion gradient will depend upon the presence or absence of carbonic anhydrase ; if enzyme is present in the tubular fluid, there will be no concentration gradient for hydrogen ion, but the electrochemical gradient will be in a direction to favor the movement of hydrogen ions from plasma to tubular urine. If the enzyme is absent, and if the intraluminal pH is of the order of 6.4, the electrochemical gradient for hydrogen ion will be in the opposite direction [based on the simplified Nernst equation, where E is designated in millivolts: $E = 61 \log (C_1/C_2)$]. This consideration might be taken to indicate that, in the absence of intraluminal carbonic anhydrase, hydrogen ion secretion would be a less probable mechanism than bicarbonate ion reabsorption. Further aspects of this problem have been discussed by Pitts (1958).

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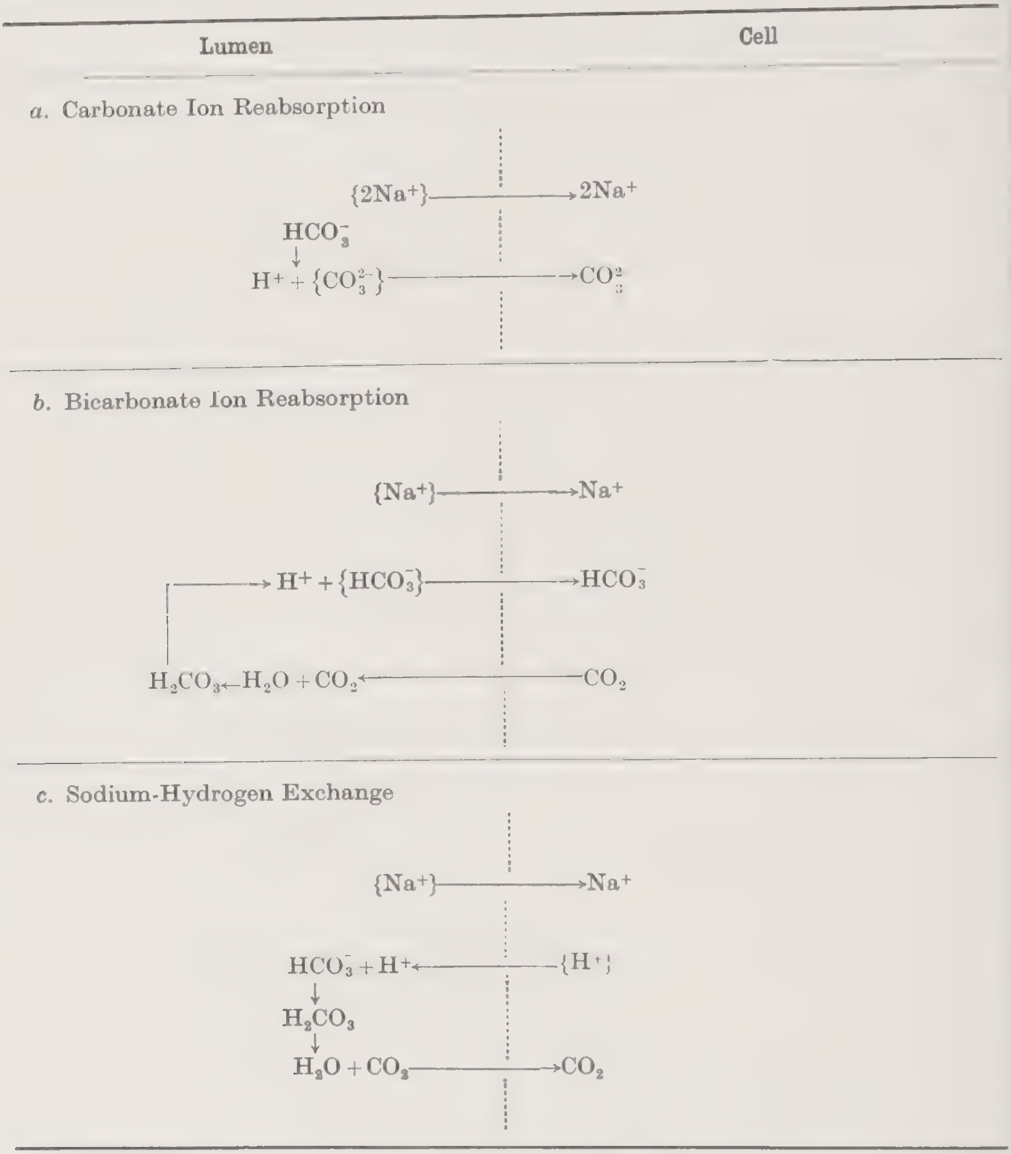
† The difference in the pK's of the two equations is due to the fact that in the former the true proton donor of the buffer pair is not CO_2, but rather H_2CO_3. In biological fluids, only a minute fraction of the dissolved CO_2 is present as H_2CO_3 (about one part in 800 at equilibrium). The former equation is generally more useful provided that this equilibrium obtains.

The net effect of proximal reabsorption at constant bicarbonate concentration is the removal of sodium, chloride, bicarbonate, and water in the proportions of plasma ultrafiltrate. Until definite evidence becomes available, the simplest mechanism appears to be the entry of hydrogen ions into the lumen in response to the electrochemical gradient established by sodium reabsorption, with carbonic anhydrase being present either within the lumen or in the cell membrane. In this type of sodium-hydrogen exchange, the movement of hydrogen ions may be a passive consequence of the transport of sodium. This implies that the membrane in this area is permeable to hydrogen ions.

2. *Cellular Mechanisms of Net Acidification*

The processes described above do not result in the net addition of acid to the tubular fluid and do not change its bicarbonate concentration. Furthermore, if the luminal pH remains 7.4, no titratable acid is formed. When the excreted urine is more alkaline than pH 7.4, the calculated value of net acidification becomes a negative quantity, and the bicarbonate concentration of the urine exceeds that of plasma. This increment in bicarbonate concentration is presumably achieved by the removal of water, sodium, and chloride in an area impermeable to bicarbonate or hydrogen ions. On the other hand, when the excreted urine is more acid than the plasma, the urine bicarbonate concentration is low, titratable acid and ammonium ions are present, and net acidification is positive.

It was believed for many years that the reduction in pH of tubular fluid, i.e., positive net acidification, was accomplished by the selective reabsorption of bicarbonate ions or of dibasic phosphate, or both. The crucial experiments of Pitts and Alexander (1945) and Schiess *et al.* (1948) established that during acidosis the total rate of filtration of "acids" (carbonic acid, dissolved carbon dioxide, and monobasic phosphate) at the glomerulus was less than the rate of acid excretion obtainable in man or the dog. It was concluded that acid must be added to the urine by the cells of the tubule. Three possible mechanisms may be considered (see Fig. 6).


a. Carbonate Ion Reabsorption

b. Bicarbonate Ion Reabsorption

c. Sodium-Hydrogen Exchange

Lumen
Cell
{2Na+} → 2Na+
HCO3-
H+ + {CO3 2-} → CO3 2-
{Na+} → Na+
H+ + {HCO3-} → HCO3-
H2CO3 ← H2O + CO2 ← CO2
{Na+} → Na+
HCO3- + H+ ← {H+}
H2CO3
H2O + CO2 → CO2

FIG. 6. Alternative Schemes of Cellular Mechanisms of Net Acidification. The initiating reactions are given by the symbols enclosed in the braces.

a. Carbonate ion reabsorption. Menaker (1948) calculated that, even if the tubules were impermeable to CO_2, reabsorption of the filtered bicarbonate as carbonate could theoretically account for the above observations. Although the concentration of carbonate in the glomerular filtrate is only about $10^{-4}M$, reabsorption of this quantity would cause more carbonate to be formed by the reaction between bicarbonate and other urinary buffers. In this way continued removal of carbonate could account for the highest observed rates of acid excretion.

The principal objection that has been raised to this proposal is that carbonate would have to be transported from a vanishingly low concentration in the distal tubular fluid, e.g. $10^{-9}M$ at pH 5, to the plasma, where its concentration is about $10^{-4}M$. The simultaneous concentrations of the hydrogen ion in these two phases would be 10^{-5} and 10^{-7} respectively. Thus, the reabsorption of carbonate would require transport from a lower concentration and against a larger gradient than would the transport of hydrogen ion from the cell into the tubular fluid.

b. Bicarbonate ion reabsorption. Menaker (1948) also pointed out that if the tubules are permeable to CO_2, urinary acidification could be affected by the reabsorption of bicarbonate ion as such. Removal of bicarbonate ions from the tubular fluid would result in a reaction between carbonic acid and other urinary buffers, converting carbonic acid to bicarbonate. The partial pressure of carbon dioxide in the tubular fluid would tend to fall. Consequently, carbon dioxide would diffuse into the urine from the cells. Thus, acid would be effectively added to the filtrate as carbonic acid. A fundamental difference between the sodium-hydrogen exchange theory (see below) and the bicarbonate ion reabsorption theory is that in the former CO_2 diffuses out of the tubule, while in the latter CO_2 diffuses into it. According to the calculations of Berliner (1957), the uncatalyzed hydration reaction would not be of sufficient magnitude to account for the highest observed rates of net acidification. Hence, if the bicarbonate ion reabsorption hypothesis is correct, the fluid within the distal tubule must have access to carbonic anhydrase.

c. Sodium-hydrogen exchange. As indicated in Fig. 6, the active reabsorption of sodium, coupled with the active secretion of hydrogen ions (against an electrochemical gradient) will result in net acidification of the tubular fluid. It is necessary in this scheme for the tubule to be permeable to CO_2, but it is not certain whether carbonic anhydrase need be available to the tubular fluid.

At present there is no conclusive evidence which gives direct support to any one of the above mechanisms in preference to the others.

E. Partial Pressure of Carbon Dioxide in the Urine

The work of Pitts and Lotspeich (1946) established that on the average the partial pressure of carbon dioxide in acid urines (p_UCO_2) is identical with that of arterial plasma (p_ACO_2). These findings have been interpreted as indicating that the renal tubules are freely permeable to CO_2. In alkaline urines, on the other hand, p_UCO_2 may exceed p_ACO_2. The elevation of p_UCO_2 in alkaline urines has been attributed to a number of causes, each operating in a portion of the kidney that has a limited permeability to CO_2: (1) delayed dehydration of carbonic acid (Pitts and Lotspeich, 1946), (2) admixture within the collecting ducts of urines with differing pH and differing buffer content (Mainzer and Bruhn, 1931; Kennedy *et al.*, 1952), and (3) retardation of carbonic acid dehydration by the presence of buffer (Kennedy *et al.*, 1957). Another possibility is that the removal of water within the collecting duct may concentrate bicarbonate, carbonic acid, and carbon dioxide equally, thus leading to an elevation of p_UCO_2. The measurement of p_UCO_2 during water diuresis has not been adequately studied. Values of p_UCO_2 less than p_ACO_2 have been reported during organic acid loading and during acute respiratory acidosis (Brodsky, 1955).

Ochwadt and Pitts (1956) showed that the infusion of carbonic anhydrase led to a reduction of the elevated pCO_2 of alkaline urine. This has been cited as evidence of delayed dehydration within the bicarbonate reabsorbing portions of the nephron and, therefore, as evidence for sodium-hydrogen exchange.

F. Ammonium Excretion

Under ordinary conditions, approximately one-third of the excess of fixed anions over fixed cations in the urine is balanced by ammonium ions. During acidosis, ammonium excretion is increased. Its presence in the urine presumably facilitates the excretion of acid by reducing the pH gradient against which urinary acidification must occur. Thus, subjects who excrete abnormally low quantities of ammonium exhibit impaired resistance to acid loads.

Ammonia is formed in the kidney principally from glutamine, through the action of glutaminase, and to a lesser extent from other amino acids. During acidosis, ammonia production increases for several days, but this increase is poorly correlated with changes in the glutaminase activity of renal tissue (Rector and Orloff, 1958). Of the ammonia produced by the kidney, only a portion is excreted in the urine (Poppel *et al.*, 1956). This fraction depends on urine pH. The nature of this relationship is consistent with the postulate that the tubule cells are permeable to free ammonia, but relatively impermeable to ammonium ions (see Orloff and Berliner, 1956).

The rate of excretion of ammonium at a given pH is not fixed, but may vary considerably. For example, potassium depletion is associated with an increase in ammonium excretion relative to pH (Clarke *et al.*, 1955; Womersley and Darragh, 1955; Milne *et al.*, 1957). Similar effects follow the administration of adrenal steroids (Seldin *et al.*, 1952; Relman and Schwartz, 1952) and may be a reflection of the accompanying potassium depletion. On the other hand, adrenalectomy impairs ammonium excretion (Wilson and Seldin, 1957). Renal glutaminase activity is increased in potassium deficiency (Iacobellis *et al.*, 1954) but unaffected by adrenalectomy (Wilson and Seldin, 1957). The exact relationship between the activity of glutaminase and the rate of ammonia synthesis has not been established.

G. Renal Response to Changes in Acid-Base Balance

In general, the most potent stimulus affecting net acidification is a change in labile anion content of the extracellular fluid, i.e., metabolic acidosis or alkalosis. Acid-base disturbances that do not primarily involve alterations in labile anion content, i.e., respiratory acidosis and alkalosis, are less effective stimuli. In man and experimental animals, mixed disturbances (i.e., both respiratory and metabolic alterations) are usually seen as a result of compensatory adjustments. Furthermore, accompanying changes in fluid balance are often observed. These complicating factors prevent an exact analysis of the relationship between plasma composition and net acidification. An increase in net acidification elevates the labile anion content of the body and thus tends to increase extracellular pH. Negative net acidification (i.e., excretion of appreciable amounts of bicarbonate) effectively reduces the labile anion content of the body and thus lowers extracellular pH.

The relationship between plasma composition and bicarbonate reabsorption has been studied intensively in an effort to determine how the adjustments described above are accomplished. Pitts and Lotspeich (1946) interpreted their findings as showing an apparent tubular transfer maximum for bicarbonate. Further studies have shown that bicarbonate reabsorption varies with the partial pressure of carbon dioxide in the blood when plasma bicarbonate is elevated, but not when plasma bicarbonate is low (see Gilman, 1958).

H. Determinants of Net Acidification during Severe Acidosis

The rate of net acidification during metabolic acidosis varies considerably depending upon the following factors:

1. In highly acid urines the titratable acid varies with the rate of buffer excretion (Pitts and Lotspeich, 1946).

2. Individual buffers vary in their ability to enhance net acidification, depending upon their acidic dissociation constants. A buffer which is only half acidified in reducing the pH from 7.4 to 5.0, e.g., creatinine, $\text{p}K_a = 5.0$, is approximately half as effective mole for mole as a buffer

which is completely acidified, e.g., phosphate, $pK_a = 6.8$ (Schiess *et al.*, 1948).

3. The pH of the urine in severe acidosis does not appear to have a fixed minimal value. More acidic urine is observed when the excretion of unreabsorbed anions, such as sulfate, is increased ; somewhat less acidic urines are seen when buffer excretion is increased. The explanation of this observation is uncertain.

I. Factors Affecting Net Acidification, Independent of Plasma Composition

Bicarbonate is excreted in the urine in association with fixed cations. Hence, the rate of bicarbonate excretion in response to metabolic alkalosis is conditioned by the simultaneous tubular activity with respect to the reabsorption of fixed cation. For example, the continuous administration of sodium bicarbonate solutions to experimental animals results in the excretion of bicarbonate and fixed cation, mostly sodium. On the other hand, when metabolic alkalosis is associated with diminished extracellular fluid volume, as may result from prolonged vomiting, the urine may be acid and bicarbonate excretion may be negligibly low (Van Slyke and Evans, 1947). It is uncertain whether this effect is mediated through changes in filtration rate, adrenal cortical activity, or other factors. Acid urine despite metabolic alkalosis is also seen in the presence of potassium depletion (see above).

In metabolic acidosis, the rate of net acidification appears to be influenced by sodium metabolism in at least two different ways. Schwartz *et al.* (1954) found that sodium depletion enhanced the net acidification response to a challenge dose of acidifying salt and interpreted their findings as indicating an adaptation of distal sodium-hydrogen exchange. A second and quite different effect can be demonstrated in experiments in which the rate of sodium excretion is acutely altered by the infusion of an osmotic diuretic (Beck, 1958) or of the sodium salt of a nonreabsorbable anion (Schwartz *et al.*, 1955). When superimposed upon either acidosis or sodium depletion, this procedure is accompanied by a marked rise in the rate of net acidification. It has been attributed to an increase in the concentration of sodium in the distal tubular urine.

Changes in the glomerular filtration rate appear to affect the processes of net acidification depending upon the underlying state of acid-base balance. In metabolic alkalosis due to sodium bicarbonate infusion, the rate of bicarbonate excretion is decreased when glomerular filtration is moderately reduced (Thompson and Barrett, 1954b). Under normal conditions or with slight metabolic acidosis, a moderate reduction in filtration rate is accompanied by little or no change in net acidification.

provided that the excretion of unreabsorbable anion makes sufficient sodium available to the distal tubule (Lauson and Thompson, 1958). In brief experiments without prior loading, net acidification falls in proportion to the reduction in filtration rate (Avedon *et al.*, 1958).

VI. Calcium

The factors affecting the renal excretion of calcium have been extensively documented in balance studies†. Renal mechanisms of calcium excretion, however, have been examined in only a few reports. This circumstance results in part from the difficulty of determining the physicochemical state of calcium in plasma. Three forms are present: protein-bound nondiffusible calcium (about 33%), diffusible but undissociated calcium complexes (about 12%) and free calcium ions (about 55%) (see Neuman and Neuman, 1958).

Hence, the filtered calcium is in two forms, undissociated complexes and free calcium ions. It has been generally assumed that the concentration of calcium in plasma ultrafiltrate is an accurate measure of its concentration in the glomerular filtrate. Toribara *et al.* (1957) have emphasized that valid results are obtained only if the ultrafiltrate is prepared at the pH of the plasma and 37°C. A discussion of the mechanism of calcium excretion by the kidney must be based primarily upon data in which both ultrafiltrable calcium and glomerular filtration rate are reported. The number of such studies is as yet very small.

In the normal dog, 0.3–1.0% of the filtered calcium is excreted (Chen and Neuman, 1955a; Freeman *et al.*, 1957). Intravenous infusion of calcium salts leads to a progressive rise in calcium excretion which may reach 20% of the amount filtered (Chen and Neuman, 1955a). The determination of calcium reabsorption under these circumstances is complicated by the fact that glomerular filtration rate usually falls in the dog during calcium infusion. Smaller amounts of calcium administered intravenously to human subjects have no effect on filtration rate (Levitt *et al.*, 1958). In two animals in which filtration rate did not fall during calcium infusion, Poulos (1957) observed that increased urinary excretion occurred simultaneously with a continuous rise in the rate of calcium reabsorption. Thus calcium excretion resembles sodium excretion in that it is responsive to the plasma level and is apparently not characterized by a maximal rate of reabsorption.

Studies on the effects of various agents on calcium excretion are somewhat complicated by the fact that the output of calcium tends to vary with urine flow (Wolf and Ball, 1949, 1950). This relationship has not been studied in detail but may account for part of the hypercalciuria observed following the administration of a number of agents. Similarly, mannitol diuresis may lead to the excretion of 20% of the filtered calcium at a time when 40% of the filtered water is being excreted (Chen and Neuman, 1955a). Thus, the rate of calcium reabsorption, in common with the reabsorption of several other ions, may depend on its concentration in the tubular fluid.

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† See Vol. II, Chapter 20.

The rate of excretion of unreabsorbable anions may also influence calcium reabsorption. Phosphate infusion may produce considerable increments in calcium excretion, exceeding 15% of filtered calcium (Jahan and Pitts, 1948; Chen and Neuman, 1955a). Sulfate infusion has even more marked effects (Wolf and Ball, 1950; Walser and Browder, 1958). The presence of increased quantities of unreabsorbed anion in the filtrate may account for the rise in calcium excretion following infusions of sodium pyruvate, lactate, hippurate, acetate, and malate during urea diuresis (Brull and Bernimolin, 1956). These salts were also observed to increase the filtrability of plasma calcium *in vitro*.

The rate of calcium excretion can be considerably increased by the administration of either citrate (Chang and Freeman, 1950) or ethylenediamine tetraacetic acid (Spencer *et al.*, 1952; Chen and Neumann, 1955a) both of which form highly undissociated complexes. Presumably, tubular reabsorption of the complex is considerably less than that of ionized calcium.

The effects of parathormone and of Vitamin D on calcium excretion are discussed in Vol. I, Chapter 14; Vol II, Chapters 20 and 21.

The reported effects of disturbances in acid-base balance upon calcium reabsorption are difficult to interpret since urine flow and phosphate excretion also varied widely (Freeman *et al.*, 1957). The effects of acetazolamide on calcium excretion are controversial (Chen and Neuman, 1955b; Freeman and Jacobsen, 1957).

VII. Magnesium

Magnesium resembles calcium in that about 35% of the amount in plasma is bound to protein (Copeland and Sunderman, 1952). Studies of the renal excretion of magnesium are scarce, due in part to analytical difficulties.

In normal subjects 3–5% of filtered magnesium is excreted (Heller *et al.*, 1953; Womersley, 1956; Hammarsten *et al.*, 1957). During magnesium deficiency its reabsorption by the tubules may become almost complete (Fitzgerald and Fourman, 1956; Barnes *et al.*, 1958). When magnesium salts are infused intravenously, both magnesium excretion and tubular reabsorption of magnesium increase sharply. As plasma magnesium is further increased, tubular reabsorption tends to become fixed. The quantity of magnesium excreted has not been observed to exceed the amount filtered (Heller *et al.*, 1953 ; Womersley, 1956 ; Hammarsten *et al.*, 1957 ; Barker *et al.*, 1957 ; Chesley and Tepper, 1958).

The relationship between acid-base balance and magnesium excretion has not been studied in detail. Acetazolamide has little effect on magnesium either before or after magnesium loading (Jabir *et al.*, 1957).

VIII. Phosphate

The inorganic phosphate of plasma at pH 7.4 consists of dibasic and monobasic phosphate in a ratio of 4 : 1. Although plasma phosphate is completely or nearly completely ultrafiltrable (Foulks, 1955 ; Neuman and Neuman, 1958), about 7% is held to be associated with calcium, presumably as $CaHPO_4$ (Davies and Hoyle, 1953 ; Gosselin and Coghlan, 1953). A smaller fraction is probably associated with magnesium (Tabor and Hastings, 1943). When plasma calcium or plasma phosphate is elevated, a nondiffusible form of calcium phosphate may appear in the plasma in significant amounts (Grollman, 1927 ; Hopkins *et al.*, 1952).

Exogenous labeled phosphate is excreted more rapidly than endogenous phosphate (Govaerts, 1948). It was originally proposed that this observation indicated that endogenous phosphate was not completely filtered at the glomerulus. However, a similar phenomenon has been observed with a number of other ions. Several additional interpretations have been offered, which do not require that the filtrability of the isotopic ions be different from that of the native ions. These include the delay time, the heterogeneity of the nephron population, and the exchange of unlabeled ions from the tubular fluid with labeled ions from the interstitial fluid (Chinard and Enns, 1955 ; Childs *et al.*, 1955).

Under normal circumstances some 5% of the filtered phosphate is excreted, but this fraction varies greatly. When protein intake is low and carbohydrate utilization high, phosphate may virtually disappear from the urine (Pitts and Alexander, 1944). In severe renal insufficiency, on the other hand, phosphate reabsorption nearly ceases (Goldman and Bassett, 1954). Phosphate is secreted by the renal tubules of the chicken (Davidson and Levinsky, 1957), but convincing evidence that secretion occurs in mammalian kidneys has not been presented.

In dogs and man the capacity of the renal tubules to reabsorb phosphate is limited, the maximal rate (T_m) being approximately 120 μmoles per minute per 100 ml of glomerular filtrate in both species (Pitts and Alexander, 1944 ; Thompson and Hiatt, 1957a). However, phosphate T_m varies considerably, so much so that its existence has been questioned (Smith *et al.*, 1943 ; Eggleton and Shuster, 1954 ; Crawford *et al.*, 1955). Conflicting results have been reported concerning the effects of alkalosis on phosphate transport. Alkalinization of the urine was associated with a reduction of phosphate T_m in the studies of Malvin and Lotspeich (1956), although no changes had been observed by Pitts and Alexander (1944). Phosphate T_m is increased by phlorizin and sulfate and decreased by glucose (Pitts and Alexander, 1944 ; Cohen *et al.*, 1957 ; Foulks, 1955). The repeated administration of phosphate results in a reduction of phosphate T_m, an effect that is probably mediated by the parathyroid glands (Thompson and Hiatt, 1957b).

IX. Sulfate

Plasma sulfate is completely or nearly completely ultrafiltrable (Goudsmit *et al.*, 1939 ; Swan *et al.*, 1956). Approximately 30 % and 10 % of the filtered sulfate is excreted in man and in the dog, respectively, but this fraction varies greatly with urine flow (Goudsmit *et al.*, 1939 ; Smith, 1951).

In the dog, progressive elevation of plasma sulfate concentration produces only a slight increase in sulfate reabsorption, which quickly attains a maximal rate of approximately 150 μmoles per minute per 100 ml of glomerular filtrate (Lotspeich, 1947). Sulfate T_m, like phosphate T_m, is subject to considerable variation. The apparent T_m also varies with the glomerular filtration rate. Reabsorption is depressed by infusions of sodium chloride, glucose, amino acids, or thiosulfate, and is increased by phlorizin (Berglund and Lotspeich, 1956a, b ; Cohen *et al.*, 1956).

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CHAPTER 10

Extrarenal Regulation with Special Reference to the Sweat Glands†

IRVING L. SCHWARTZ

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For the most part this chapter will describe nonrenal exchanges of elementary body constituents between man and his environment. The scope of the term " regulation " has been narrowed by minimizing discussion of transfers within the organism and by limiting citation of studies on lower animals‡ to those which seemed to point toward principles of general interest, or for which there were either no human counterparts or markedly differing ones. Both the selection of material and the variable emphasis given to different topics have necessarily been somewhat arbitrary, as has been the omission of references to some original papers, especially when included in cited reviews or monographs.

The major extrarenal organ systems for excretion and therefore, in a limited sense, for regulation of the mineral and water content of the body are the skin and its appendages, the gastrointestinal tract, the lungs, the uterus in menstruation and at parturition, and the mammary glands during lactation. The glandular and other epithelial structures of the nose,† mouth, pharynx, respiratory passages, eyes, ears, and genital tracts are not significant avenues of mineral and water loss from the body.

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† This work was done in part under the auspices of the United States Atomic Energy Commission.

‡ The comparative physiology of excretion, renal and extrarenal, has been reviewed recently (Beadle, 1957; Martin, 1958).

I. Gastrointestinal Tract

Under normal circumstances the constituents of secretions of the hepatobiliary system, the pancreas, as well as of the salivary glands,‡ stomach, and intestines,§ enter the gastrointestinal tract at particular levels and are reabsorbed at lower sites. The absorption of water appears to be entirely secondary to the processes, passive and active, that determine the direction and quantity of all solutes being transferred. The nature, modifying influences, and ultimate effectiveness of these processes in bringing about more or less complete absorption of ingested substances and secretion or resecretion of previously absorbed solutes are treated generally in Vol. I, Chapter 8, and specifically in Volume II (the special case of iron absorption being considered in Chapter 30).

The dietary intake of minerals and water is largely a fortuitous consequence of food intake in general, as determined by cultural, economic, geographical, psychological and physiological factors. In man, the physiological influences appear to be readily modified and often nullified by factors in the other categories. However, the literature on free choice feeding indicates that lower animals and man are endowed with at least potential appetites for specific nutrients, particularly minerals, which guide them to selections appropriate to their needs (Richter, 1942–1943 ; Lepkovsky, 1948 ; Denton, 1957). Behavior contrary to this principle has been noted by Kon (1931), Scott (1946), and recently by Yudkin (1956), who cites observations of anomalous food selection in animals with sodium and magnesium deficiency. For example, salt-deprived adrenalectomized animals freely chose a sugar solution in preference to a salt solution and died sooner as a consequence of this choice. This suggests that the greater palatability of the sugar solution had overridden the more appropriate regulatory influences that had led the same animals to select a salt solution in preference to a water solution. The magnesium-deficient animal responds even more inappropriately to his nutritional needs, avoiding the selection of diets containing magnesium.

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† Certain marine birds are equipped with nasal glands which assist in the adaptation of the animals to general hyperosmotic stress. In response to loads of salt and sucrose the cormorant, *Phalacrocorax auritus*, can secrete through its nose at rates as high as 0.1 ml/min per kilogram of body weight a hypertonic fluid containing 500–600 meq/liter of sodium chloride, approximately twice the concentration of the most concentrated urine the bird can produce (Schmidt-Nielsen *et al.*, 1957, 1958; Schmidt-Nielsen and Sladen, 1958).

‡ A significant loss of salivary constituents (Table 50, Spector, 1956) would be most unlikely even in humans with a spitting habit. However, experimental parotid fistulas in ruminant animals, e.g. sheep, can lead to daily losses of two or more liters of alkaline saliva (Coats *et al.*, 1956), followed in turn by sodium depletion, adrenal hyperfunction (Denton and MacDonald, 1957 ; Goding and Denton, 1957), and a striking change in saliva composition from a predominantly sodium to a predominantly potassium secretion (Denton, 1956).

§ Abnormal gastrointestinal mineral excretion in diarrheal states is reviewed in Vol. I, Chapter 8. Vomiting of intestinal content (Table 51, Spector, 1956) presents a picture similar to diarrhea with respect to the mineral balance of the body. However, vomiting of gastric juice, as for example after pyloric obstruction, results in loss of hydrochloric acid from the body followed by elevation of the bicarbonate ion concentration and the pH of the extracellular fluid, the latter event tending to be compensated initially by retention of carbonic acid and ultimately by proportionate excretion of both bicarbonate and carbonic acid.

Magnesium deficiency also causes a decreased intake of food in general with no change or considerable increase in the activity of the affected animal (Lepkovsky, 1948). Calcium, phosphorus, and sodium deficiencies also suppress appetite for total calories, but specific appetites for the deficient elements are increased (Lepkovsky, 1948). A more detailed discussion of the effects of mineral deficiency or surplus on general and specific appetites is beyond the scope of this chapter, but the topics have been covered in the reviews cited (Richter, 1942–1943 ; Lepkovsky, 1948 ; Yudkin, 1956). Also, concise reviews of the central regulation of feeding behavior (Mayer, 1957) and the mechanism of thirst (Strauss, 1957) have appeared recently.

II. Mammary Gland

The secreting unit of the mammary gland is the alveolus which consists of a basement membrane, myoepithelial cell layer, and internal columnar epithelium. The alveolus empties into the alveolar duct and, in turn, into lobular and lactiferous ducts which open individually on the apex of the nipple. The mechanism of secretion resembles that of the apocrine sweat glands† in that there is rupture of the luminal surface of the alveolar epithelial cells with discharge of cytoplasm including the specific components of milk. In addition, a merocrine‡ secretion differing slightly in composition from extracellular fluid may occur within the alveolus or ducts and mix with a primary milk secretion. An increase in rate of such a merocrine secretion relative to a primary apocrine milk secretion, especially after the latter has attained maximal values, would explain the rise in sodium and chloride concentrations and the concomitant fall in potassium and lactose concentrations observed as lactation advances (Barry and Rowland, 1953). In humans, during continued lactation, the daily output of milk increases to maximum values of 1 to 3 liters per day. Milk secretion stops when the pressure in the alveoli rises above 25 to 50 mm Hg ; hence frequent emptying increases milk production. Some of the properties of milk are common to all mammals. Freezing point measurements show it to be isotonic with blood. Its specific gravity ranges from 1.025 to 1.035 ; its pH ranges from 6.6 to 7.0.

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† Attention has been called to tubular structures in the human breast which resemble apocrine sweat glands and communicate with the lactiferous duct system (Bunting, 1948). The cells of these structures contain granules which react with iron stains. Similar iron granules are not seen in resting human mammary epithelium but have been demonstrated in the mammary epithelial cells of rats and mice (Rawlinson and Hankinson, 1948). The latter finding is of interest in connection with earlier proposals of a developmental as well as a functional relationship between the mammary and apocrine sweat glands.

‡ The terms " merocrine " or " exocrine " refer to glands or gland products that do not involve destruction of the secreting cells. The term " holocrine " refers to a secretion which consists of altered cells of the gland itself ; the term " apocrine " refers to a gland the cells of which lose part of their protoplasmic substance when functioning.

The major mineral constituents of milk are calcium, sodium, potassium, magnesium, chloride, phosphorus, and sulfur ; the minor components are iron, copper, zinc, manganese, cobalt, iodine, and fluorine. The general biochemistry and physiology of milk secretion has been reviewed by Petersen (1944) and recently by Folley (1956).

TABLE I

MAJOR MINERAL CONSTITUENTS OF HUMAN, COW, GOAT, AND RAT WHOLE MILK[a,b]

Constituent	Human	Cow	Goat	Rat
Calcium	0.3	1.25	1.3	3.49
Phosphorus	0.15	0.96	1.06	2.72
Magnesium	0.04	0.12	0.16	0.31
Sodium	0.15	0.58	0.41	0.76
Potassium	0.55	1.37	1.80	1.68
Chloride	0.43	1.03	1.60	1.17
Sulfur	0.14	0.3	0.16	—
Iron	0.0015	0.001	0.0005	0.007
Copper	0.0004	0.0003	0.0004	0.007
Zinc	0.0053	0.0038	Trace	—

[a] Grams per liter.

[b] Compiled from Shohl, 1939 ; Stearns, 1939 ; Barry and Rowland, 1953 ; Spector, 1956.

The rate of secretion of milk and the concentrations of individual components depend on nutritional, endocrine, nervous, and other factors, such as the frequency and completeness of milk withdrawal, the duration of lactation, and the presence or absence of mastitis. Mineral concentrations vary greatly among individuals of a single species and even among different samples from a single individual. This variability should be kept in mind in reviewing the representative values for the major mineral constituents of human, cow, goat, and rat whole milk given in Table I.

The conspicuously higher mineral content in rat milk as compared with human milk supports a thesis, based on studies of many species, that the concentration of milk solids is related directly to the rapidity with which the young animal grows. The relative proportions of inorganic and organic phosphorus (chiefly as phosphoprotein and phospholipid) and of diffusible (i.e., dialyzable through ordinary cellophane membranes) and nondiffusible forms of calcium and phosphorus vary in milks of different species. In human milk half of the calcium and one-fourth of the phosphorus is diffusible, and the phosphorus is divided approximately evenly between inorganic and organic forms.

Although negative balances of sodium, potassium, chlorine, sulfur, magnesium, and particularly of calcium and phosphorus, occur in the nursing mother despite a generous mineral intake and vitamin D supplement, the reverse is true of the second and third trimesters of pregnancy when, if intake is adequate, mineral reserves are accumulated by the mother as a result of positive balances in excess of fetal requirements. The negative calcium balance of the lactation period is not necessarily a simple consequence of the calcium loss in milk since it may persist for as long as a year after lactation has stopped. It is noteworthy that women and lactating animals, such as the dairy cow, can sustain a temporary depletion of one-fifth or more of the body calcium store without evident deleterious effects (Shohl, 1939 ; Hegsted, 1955).

III. Lacrimal Gland

The daily secretory output of the lacrimal gland is minute normally and does not become significant in the mineral economy of the body even when lacrimation is maximal. The chief known mineral constituents of tear fluid are chloride, sodium, and potassium, all of which have been found to be excreted at concentrations independent of the rate of lacrimal secretion (Thaysen and Thorn, 1954). Chloride and potassium are more concentrated in tears than in serum, but the concentration of sodium, the concentration of bicarbonate, and the pH are the same in the two fluids. Freezing point measurements show tears ($\Delta^{\circ}C = -0.55$) to be isotonic with extracellular fluid. Otherwise, very little quantitative information is available concerning the chemical, and particularly, the mineral composition of tears.

IV. Genitourinary Tract

There have been some analyses of the mineral content of the mucous secretions of the body including those of the genitourinary tracts (Huggins, 1945), but these data have slight, if any, relation to mineral metabolism in the context of this chapter. For those interested, a listing of the sodium, potassium, chloride, calcium, magnesium, phosphorus, and trace metal content of the prostatic fluid and semen of man, dog, horse, rabbit, sheep, cattle, and swine may be found in the " Handbook of Biological Data " (Table 48, Spector, 1956).

Menstruation occurs periodically in all Old World Primates throughout the period of sexual fertility except during pregnancy and lactation. In women, the interval between menstrual periods averages 28 ± 3 days. The loss of blood in each period ranges from 10 to 200 grams, a significant amount in view of the very limited capacity for iron absorption by the gastrointestinal mucosa. With pathological increase in menstrual and/or intermenstrual hemorrhage, negative iron balance increases and a chronic hypochromic microcytic anemia develops.

V. Respiratory Tract

The lungs are responsible for the excretion of the major end product of metabolism, carbon dioxide. The loss of carbon dioxide and water vapor during the respiratory gaseous exchange is discussed in the section on insensible perspiration (*vide infra*) and in Vol. I, Chapter 7. There are no significant mineral losses from the respiratory tract per se. Uncompensated acidosis and alkalosis, respiratory or metabolic, affect the internal distribution and renal excretion of electrolytes (Volume I, Chapter 9), but the effects of disturbed acid-base balance on extrarenal excretory processes appear to be minimal (*vide infra*).

VI. Skin and Skin Appendages

A. Uptake

In all animals the skin functions as a sense organ and as an envelope, protecting underlying structure from injury. In lower forms of life, the skin serves as an important instrument of respiration, alimentation, and excretion, but, in general, these functions become less important when the permeability of the skin decreases, as it does with ascent along the phylogenetic scale. In higher animals, the skin operates as a selective barrier, generally preventing the entry of matter from the environment and selectively facilitating the passage from bloodstream to exterior. The skin may also serve as a long-term depot for certain substances, such as iron (in the rat), which may be very slowly released either externally or internally. The horny layer of the epidermis and its overlying accumulation of triglycerides, cholesterol, cholesterol esters, and waxes are sites of resistance to the passage of water and electrolytes ; but there is evidence that the site of greatest resistance is the transitional layer between cornified and noncornified epidermis, which be haves like a negatively charged and, therefore, as a primarily anion-impermeable membrane. The cation permeability of the skin decreases in the following order : potassium, sodium, lithium, calcium, magnesium, aluminum : the anion permeability, such as it is, decreases as follows : iodide, chloride, nitrate, sulfate, citrate, salicylate (Calvery *et al.*, 1946 ; Lobitz, 1954 ; Rothman, 1954).

Skin appendages facilitate absorption through the skin. Heavy metal compounds, sulfonamides, and dyes enter human and guinea pig skin via the sebaceous glands and side walls of hair follicles without penetrating the epidermal barrier either from its external or internal side (MacKee *et al.*, 1945).

Metallic mercury, in particular, readily traverses the pilosebaceous glands and may be seen in a sweat gland orifice or duct cell but never in a tubule cell or lumen. The absorption of lead, tin, copper, arsenic, bismuth, antimony, and mercury depends on the formation of lipid-soluble complexes with the fatty acids of sebum.

Substances which are both lipid soluble and water soluble penetrate the epidermis and all skin appendages rapidly. The well-documented absorption through intact skin of lipid-soluble metal compounds, although highly significant in pharmacology, therapeutics, and toxicology, is not important in the normal physiologic regulation of the mineral content of the body. Aqueous solutes do not penetrate hair follicles, apocrine or sebaceous glands, or the ducts of tubules of the sweat glands. Tritium and deuterium, presumably in the vapor phase, penetrate the nonsweating skin and/or its appendages from the outside at approximately the same rate at which water moves in the reverse direction as insensible perspiration (Szczesniak *et al.*, 1951 ; Pinson, 1952). There is no evidence that electrolytes in water solution can traverse human skin in more than trace amounts. However, radioactive strontium has been shown to penetrate intact shaved rat skin from aqueous solutions of $Sr^{89}Cl_2$ (Loeffler and Thomas, 1951) ; and iodine, when applied to the unabraded skin of pregnant guinea pigs and rabbits for 18 to 24 hours, was found to have accumulated in significant concentration in the fetus (Miller and Selle, 1949), confirming earlier disputed evidence of dermal penetration by iodine (Calvery *et al.*, 1946).

Substances that penetrate the skin slowly or not at all can be made to do so by the use of a direct current to initiate electroendosmotic bulk flow of solution as well as electrophoresis of individual charged solutes. Other general means of promoting percutaneous penetration include use of lipoid vehicles, organic solvents, keratolytic agents, and various chemical and physical agents by which the skin can be injured and/or functionally modified (Calvery *et al.*, 1946).

The uptake of electrolytes by the skin of amphibians has been studied by physiologists interested in ion transport processes and is reviewed in Vol. I, Chapter 6.

B. Insensible Perspiration

The total insensible weight loss of the body is due to the continuous discharge of evaporated water from the skin surface and lungs and to the fact that, in the usual gaseous exchange, the mass of CO_2 excreted exceeds the mass of O_2 absorbed.† This total insensible loss amounts to about 500 to 800 gm per day for adult humans resting under comfortable conditions without manifest sweating (Benedict and Root, 1926; Rothman and Schaaf, 1929; Rothman, 1954; Kuno, 1956). Reported variations ranging from 450 to 1900 gm per day reflect extreme differences in body size, metabolic rate,‡ barometric and water vapor pressure (Hale *et al.*, 1958), and, undoubtedly, different states of sweat gland activity among the subjects studied.

The respiratory component of the total weight loss in normal resting subjects has been found to vary from 138 to 468 gm per day, a range of variation which results from differences in the volume of respiration, the humidity of the inspired air, and the saturation of the expired air. Water loss is the dominant factor in the respiratory component, but the gaseous exchange may be equally important in some circumstances.†§ The respiratory and cutaneous components of the insensible weight loss appear to be related reciprocally, increases in one component being associated with decreases in the other (Adachi and Ito, 1934), but there are no clues as to how this relationship is brought about.

From 50 to 70% of the total insensible weight loss is due to "insensible perspiration," that is, evaporation of water from the skin surface§ (Rothman, 1954; Kuno, 1956). This cutaneous water loss can be subdivided into two processes. The first is a continuous passage through the epidermis and discharge from the skin surface. This process is little affected by ambient temperature and takes place independently of sweat gland function, since it is found both in individuals with congenital absence of sweat glands (Richardson, 1926; Sunderman, 1941; Felsher, 1944; Upshaw and Montgomery, 1949) and in normal subjects in whom the sweating function has been eliminated by atropinization (Rothman, 1954) or by introduction of formaldehyde into the skin (Pinson, 1942). The second process is a low-grade secretion of sweat which takes place at temperatures as low as 20°C, and can be seen microscopically or by sensitive indicator methods (Randall, 1946; Thaysen *et al.*, 1952; Dole and Thaysen, 1953) but which cannot be visualized grossly because of immediate evaporation at the duct orifice. The process of transepidermal diffusion is the most important component in resting subjects at comfortable temperatures, e.g., about 25° C, (well below the critical temperature for general sweat outbreak), accounting for 90% or more of the cutaneous water loss. On the limited areas of the body surface where sweating is readily evoked by psychic stimuli, such as the palm of the hand, sole of the foot, and axilla, the ratio of the sweating component to the transepidermal diffusion component is variably but considerably greater. With increased activity and with rising environmental temperature, the diffusion component increases, but the sweating component increases at a greater rate until it is no longer "insensible." Under conditions of work and heat stress, the diffusion component, although much increased, becomes unimportant in relation to the secretory component.

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† Since the molecular weight of O_2 is 73% of the molecular weight of CO_2, a net loss of body mass occurs when the respiratory quotient (R.Q.) exceeds 0.73. At an R.Q. of 0.82, for example, the weight loss due to gaseous exchange is about 70 gm per day, which may be equal to the weight loss due to the respiratory excretion of water when the humidity is high and the rate of alveolar ventilation low.

‡ Benedict and Root (1926) and Benedict and Benedict (1926) showed the total insensible water loss of the body to be linearly related to the metabolic rate under basal conditions. The proportionality held also under nonbasal conditions, providing sweating was held in abeyance (Jores, 1930a, b; 1931). Thus, at temperatures below the critical point for outbreak of sweating, the heat eliminated from the body by the evaporation of water is a relatively fixed fraction (21–28%) of the total loss of heat (Soderstrom and Du Bois, 1917).

§ The CO_2–O_2 exchange of the skin amounts to only 0.5–1.0% of the cutaneous component of the total insensible weight loss of the body.

The rate of insensible perspiration is modified by the tonicity of the body fluids (Gilman and Barbour, 1933) and varies considerably for different regions of the skin, but the pattern is consistent in different individuals (Burch and Sodeman, 1943; Sodeman and Burch, 1944). Kuno (1956) has distinguished three arbitrary zones according to the rate of output. Zone I, which includes only the palms and soles, was found to have the highest output; Zone II, which includes the forehead, chest, and anterior surface of the neck was intermediate; and Zone III, which includes all the remaining regions of the body, was found to have the lowest output. Although recent work suggests that the mechanism of transepidermal water transport is simple diffusion (Pinson, 1942, 1952; Kuno, 1956), dependent on a vapor tension gradient across a barrier close to or identical with the stratum granulosum (Buettner, 1953), Rothman (1954) has summarized older contrary evidence and has advanced the idea that water derived from the cellular dehydration associated with keratinization contributes significantly to the transepidermal insensible loss.

It is claimed, on the basis of studies conducted in the apparent absence of sweat secretion, that there is an appreciable transepidermal diffusion of sodium, potassium, chloride, soluble nitrogen compounds, and sulfate-sulfur, but not of calcium or phosphorus (McCance, 1936, 1938 ; Freyberg and Grant, 1937). It is probable that much of the solute found under the conditions reported was derived from invisible sweat secretion, surface epithelial cells, and/or sebaceous secretion. In any case, whatever the routes, the mineral losses through the nonsweating skin are exceedingly small (Hancock *et al.*, 1929 ; Whitehouse *et al.*, 1932 ; Vasti, 1932 ; Arn and Reimer, 1950 ; Dahl *et al.*, 1955).

C. Eccrine Sweat Glands

1. *Structure, Distribution, Mechanism of Sweat Expulsion*

The eccrine glands are tubular structures, the coiled secretory portion of which is situated deep in the dermis and joins the duct in the mid-dermis. The duct traverses the epidermis in corkscrew-fashion to open on the skin surface without relation to hair follicles. On the palms of the hand and soles of the feet, but nowhere else in the body, the duct opens on epidermal ridges. The lengths of the secretory coil and of the duct are approximately equal. The coil is composed of one or two layers of epithelial cells with intracellular and intercellular canaliculae, surrounded in turn by spindle-shaped cells containing smooth muscle elements and a basement membrane. The duct has a double or triple layer of somewhat flattened cuboidal epithelial cells and a hyaline membrane lining its lumen, in addition to an external myoepithelial layer and basement membrane. Glycogen, lipid, alkaline phosphatase, and cholinesterases are demonstrable within the cells of the coil. The glycogen in these cells is depleted after secretory activity (Bunting *et al.*, 1948 ; Shelley and Mescon, 1952 ; Rothman, 1954 ; Hellman, 1955 ; Kuno, 1956).

The absence of anatomical evidence of a filtering bed in the sweat gland coupled with the striking difference between the composition of sweat and plasma ultrafiltrate and the fact that sweat may exert a pressure of 250 mm Hg, well above the systolic arterial pressure, leaves little doubt that the gland produces a true secretion. Periodic unsustained contractions of the myoepithelial fibers around the tubules probably account for a minor rhythmic fluctuation in the secretory pressure during glandular activity, as well as a rhythmic rise and fall of the column of sweat that can be observed within the duct of a " resting " gland (Lobitz and Mason, 1948b ; Kuno, 1956). Sulzberger *et al.* (1950) found that external application of cationic quaternary ammonium compounds tended to increase whereas application of anionic detergents tended to reduce the rate of sweat flow. On the basis of these observations, it was suggested that the gland outlet was electrically negative to the deeper portions of the duct and, therefore, that the gradient of potential along the duct must be considered among the factors promoting movement of water and electrolyte. Direct measurements of the potential difference between distal and proximal portions of the duct were not reported.

Embryologically the eccrine glands arise from epidermis without relation to hair follicles. In man and some species of apes the eccrine glands are distributed over the whole body surface. In other mammals the eccrine glands are found only on hairless regions, such as the footpad of the dog and cat, or are entirely absent, as in the horse. There are somewhat more than two million sweat glands in adult humans (Kawahata and Sakamoto, 1951 ; Kuno, 1956). The numerical density of glands is greatest on the palms and soles, next on the head, and less on the trunk and extremities. However, the regional distribution of gland density, size, and functional power shows great racial and individual variation (Kuno, 1956).

2. *Stimulation and Control of Secretion*

Eccrine sweat glands are under the control of cholinergic postganglionic fibers of the sympathetic nervous system and may be activated neurogenically as an axon, intrasympathetic, spinal, bulbar, hypothalamic, or cortical reflex. Non-neurogenic sweating, i.e., direct activation of sweat glands, may result from the action of local heat or cholinergic and other chemical agents.

Eccrine sweat glands become active in response to intradermal injections of as little as 10^{-3} to 10^{-4} micromoles of acetylcholine in 0.01 ml of saline (Conn and Rothman, 1941 ; Chalmers and Keele, 1952). Such responses to cholinergic agents, as well as general reflex sweating, are influenced by environmental temperature and disappear after glandular denervation. Rothman (1954) has suggested that the innervation serves among other things to maintain a tonic " conditioning " of the glands which is reflected in their capacity to respond to non-nervous stimuli. An increase in neurogenic tonus could account for the striking increase in secretory capacity observed in association with seasonal or geographic change from a cool to warmer climate, or conversely, loss of neurogenic tonus could account for diminution or loss of responsiveness to direct chemical stimulation observed after denervation but before degeneration of the glands. Also, variation in neurogenic tonus could underlie the excessive responsiveness of the sweat gland to sensory as well as thermal stimuli observed in hyperthyroidism, pulmonary tuberculosis, convalescence, and debilitated states in general ; hypermetabolism alone does not explain the functional change, especially in the latter conditions.

Changes in sweating function† in response to manipulations of the extracellular fluid tonicity and composition have been described (Amatruda and Welt, 1953), but it is not known whether the effects are exerted on neurones or directly on secretory cells, nor even whether the controlling factor is an intensive variable (osmotic pressure, hydrogen ion or other specific ion activities and/or distributions) or an extensive variable (volume).

Human and animal sweat glands are also stimulated by intradermal injection of epinephrine and by the direct application of heat. These responses have no physiological significance but are of singular interest in connection with the evolution of mammalian thermoregulatory sweating (Kuno, 1956). Under physiological conditions the secretion of sweat in response to nonneurogenic stimuli is too meager to have any significance in chemical or thermal homeostasis, and therefore there has been little study of the chemistry of sweat produced following local stimulation of the glands. The general pharmacology of sweating has been reviewed recently by Randall and Kimura (1955).

Cortical connections of the sweat pathways account for " mental " or " emotional " sweating which is elicited initially on the palms and soles, and secondarily over the entire body surface, if the cortical stimulus is sufficiently intense and sustained. Impulses from the cerebral cortex are not necessary for the elicitation of generalized reflex sweating but apparently initiate the sweating associated with muscular excercise which occurs prior to a rise in rectal temperature.

The hypothalamic sweating centers coordinate the participation of the sweat glands in thermoregulation, responding both to the central blood temperature (Magoun *et al.*, 1938; Clark *et al.*, 1939; Ranson, 1940a, b) and to signals from peripheral receptors sensitive to heat flow through the skin (Randall *et al.*, 1948; Kerslake, 1955). The palms and soles, which secrete promptly on psychic (mental, emotional, sensory) stimulation, but not in response to minimal or moderate heating, do respond to intense and sustained thermal heating. Although considerable individual variation occurs, the axillary glands and glands of the forehead behave in an intermediate fashion, responding somewhat more slowly to thermal stimulation than the glands of the body surface in general and somewhat more slowly to psychic stimulation than the glands of the palms and soles. Sensory stimuli evoke general sweating much more readily in a warm than a cold environment, acting synergistically (Kuno, 1956) with thermal stimuli, possibly another example of what Rothman (1954) has called "conditioning." Thus, eccrine glands in all locations have the capacity to respond to both psychic and thermal stimuli, but with different thresholds and different intensities.

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† There is evidence (Montuori, 1912; Dontas, 1939; Amatruda and Welt, 1953; Kuno, 1956) that the threshold of the thermoregulatory center is raised with extracellular hypertonicity and lowered with hypotonicity, that the sweating rate decreases with hypertonicity and increases with hypotonicity, and that acidosis reduces the latency and increases the intensity of the response of the sweat glands to appropriate stimulation.

3. *The Rate of Sweat Production*

Maintenance of thermal equilibrium requires that the rate of sweat secretion be adjusted to the need for evaporative heat loss as determined by metabolic heat production and radiant, conductive, and convective heat exchange between the body and its environment. Quantitatively, the relation between the sweating rate and the requirement for evaporative heat loss is modified by the state of acclimatization (Bass *et al.*, 1955) and the functional capacity of the sweat glands (Thaysen and Schwartz, 1955).

At light indoor activity in warm weather (average temperature, 29°C), a typical 65-kg man will produce from 2 to 3 liters of sweat per day. However, under conditions of severe exertion and elevated ambient temperature (35–40°C), the sweating rate can increase to from 8 to 15 liters per day, or for short periods, to over 2 and as high as 4 liters per hour (Hunt, 1912; Moss, 1923; Dill, 1938; Eichna *et al.*, 1945, 1950; Ladell, 1945, 1955; Molnar *et al.*, 1946; Adolph *et al.*, 1947; Machle and Hatch, 1947; Robinson, 1949; Winslow and Herrington, 1949; Mitchell and Edman, 1951; Rothman, 1954; Robinson and Robinson, 1954a; Bass *et al.*, 1955; Kuno, 1956). Either work alone or heat alone can, if intense enough, bring about maximal sweating rates, although the maximal rate of secretion is more readily provoked by the former. Increase in humidity increases the maximal sweating rate, presumably because evaporation is suppressed and the skin temperature is higher in humid than in dry heat.

The sweating rate is increased by negative salt balance which also lowers the threshold for activation of reflex sweating (*vide supra*). Conversely, the sweating rate is decreased by positive salt balance, although these relationships are modified by the concomitant state of hydration (Ladell, 1955; Pearcy *et al.*, 1956; Robinson *et al.*, 1956). Superimposition of dehydration will reduce the sweating rate at any level of salt balance, the decrease in rate being steepest in maximal salt but not water deficiency, in which circumstance the initial or predehydration sweating rate is highest (*vide infra*). Dehydration alone will not reduce the sweating rate until the negative water balance exceeds 2.5 liters (Ladell, 1955).

There is considerable variation in the sweating rate from individual to individual under similar conditions of ambient temperature, humidity, air movement, and exercise. However, this individual variation is much greater in cool than in warm climates, again presumably because of the dependence of sweating rate on skin temperature which exhibits much less variation in the heat than in the cold.

The sweating rate is closely related to the skin temperature under conditions of constant exercise; but with increasing exercise under fixed environmental conditions, the sweating rate rises with deep (rectal) temperature although skin temperature remains constant (Burton, 1934; Winslow and Gagge, 1941; Robinson, 1949). Clearly, therefore, peripheral and deep temperature are involved in the control of the sweating rate. In a recent study, Kerslake (1955) has shown that the sweating rate may be predicted from the temperature of the skin surface and the heat flow through the skin. He has suggested, therefore, that the sweating rate is controlled by the temperature of hypothetical subepidermal receptor nerve endings, superficial to which the thermal resistance is constant. Earlier work on the variability of sweating rates and on the factors controlling them has been summarized in several reviews (Sundstroem, 1926; Houghton *et al.*, 1931; Dill, 1938; Lee, 1940; Adolph *et al.*, 1947; Machle and Hatch, 1947; List, 1948; Winslow and Herrington, 1949; Newburgh, 1949).

4. *Sweat Composition*

Eccrine sweat is a dilute aqueous solution with a specific gravity ranging from 1.001 to 1.008 and a freezing point depression ranging from one tenth to nine tenths and averaging about one half of that of blood plasma (Schwenkenbecher, 1929; van Heyningen, 1951). Ladell (1945) has stated that sweat secreted near maximal rates may approach isotonicity with the extracellular fluid. The osmotic pressure of sweat is highly correlated with the salt concentration, NaCl accounting for an average of 79%, lactic acid for an average of 12%, and urea for about 3% of the total osmotic activity. The solid content of sweat ranges from 0.3 to 1.0%, about half of which is inorganic, and half organic. There are many conflicting reports in the literature concerning the concentration and rates of excretion of solutes in sweat, owing to the multitude of factors that determine sweat composition and the lack of uniformity of experimental conditions and collection techniques. Methods of induction, collection, and analysis of sweat have been critically surveyed by Robinson and Robinson (1954a, b), van Heyningen and Weiner (1952a), van Heyningen (1951), and Kleeman *et al.* (1953). In most studies sweat constituents have been measured by analysis of samples allowed to accumulate on the skin within a water impermeable bag or chamber. Dill *et al.* (1938), Mickelson and Keys (1943), and van Heyningen (1951) found that sweat collected in this manner is more concentrated than sweat collected under usual natural conditions which permit rapid, if not immediate, evaporation; Johnston *et al.* (1946) and Ladell (1948) reported no such differences. In any case, it is reasonable to expect that the fluxes of solutes and water through the skin sweating under natural conditions will differ from the fluxes through the unventilated saturated skin within a glove or chamber. Techniques involving collection of sweat directly from duct outlets in micropipettes are subject to error due to evaporation, as are techniques involving scraping of sweat off the skin surface. Such error is eliminated under conditions of 100% relative humidity, but these conditions are subject to the same disadvantage and uncertainties that have already been cited in connection with collections in an impermeable bag.

Sweat constituents have been determined indirectly in balance experiments by controlling intake and subtracting output in urine and feces. Without independent direct measurement of cutaneous excretion, the validity of this method depends on the hazardous assumption that the subject was in precise metabolic equilibrium with respect to the constituent under observation.

Experiments in which sweat is allowed to evaporate from the skin as it is secreted best simulate natural environmental conditions (Hancock *et al.*, 1929; Lee *et al.*, 1941; Mickelson and Keys, 1943; Robinson *et al.*, 1950a; Weiner and van Heyningen, 1952a; Robinson and Robinson, 1954b). The water vapor is condensed (Neuman *et al.*, 1941; Burch and Sodeman, 1943), absorbed (Pinson, 1942; Kuno, 1956), or measured in an infrared gas analyzer (Albert and Palmes, 1951), or more often, the water content of the sweat is approximated by simply determining the change in weight of the subject with correction for intake and excretion of fluids and solids during the experimental period. The deposit of sweat solids that accumulates on the skin and in the clothing is then collected and analyzed independently.

Unless otherwise specified, composition of sweat as collected includes nonsweat water derived from the diffusion component of insensible perspiration and nonsweat solutes which also have diffused to the surface from subepidermal tissue or which have been leached out of epidermal cells.

a. Sodium and Chloride. Sodium, chloride, and water are the only substances which may be excreted in sweat in quantities sufficient to disturb homeostasis and seriously impair the economy of the organism. Water and salt deficits incurred in the course of profuse prolonged sweating must be replenished before reduction in the volume of extracellular fluid brings about significant impairment of the circulation, thus decreasing the efficiency of heat dissipation and, in the face of continuing or increasing heat load, initiating a vicious cycle of dehydration, cardiovascular inefficiency, and heat retention (Bass *et al.*, 1955).

Since sweat is hypotonic, its unrestored loss first leads to an increase in the osmotic pressure of the extracellular fluid, an effect which tends to be compensated by withdrawal of intracellular water, by production of hypertonic urine, and according to Kuno (1956), by temporary salt storage in the skin. Replacement with hypertonic salt solutions exaggerates both the extracellular and intracellular dehydration ; replacement with water alone results in lowering of the osmotic pressure of the body fluids and, in conjunction with the progressively developing salt depletion, leads to the signs and symptoms of water intoxication.

Thirst is a poor index of water requirements during a bout of sweating since it lags considerably behind the developing negative water balance and disappears before the water deficit has been replenished (Adolph *et al.*, 1947 ; Kuno, 1956). Water deficits of from 2.5 to 4 liters (approximately 5% of body weight) can develop in men drinking water ad libitum and can be tolerated without signs or symptoms of dehydration. With a further deficit of 1 to 2 liters of body water, signs and symptoms develop and quickly proceed to dehydration exhaustion.

An increased salt appetite is a poor index of salt requirements. Nevertheless the salt craving of adrenalectomized animals and of about 20% of humans with Addison's disease is well documented (Strauss, 1957), and very likely some enhancement of salt appetite develops in response to salt depletion following sweating.

Even if it were possible to know in advance the precise sweating rates of humans under various conditions of work and environmental temperature, it would not be possible to predict salt requirements because of the extreme variability and multiplicity of influences on the salt concentration of sweat. In a comprehensive review of the literature of the chemical composition of sweat, Robinson and Robinson (1954a) found reports of the sodium concentration of thermal sweat varying from 5 to 148 meq/liter depending on sweating rate, skin and deep (rectal) temperature, anoxia, work, acclimatization (repeated exposures to heat and work stresses), duration of sweating in a single exposure, skin site, plasma concentration, adrenal cortical activity, changes in salt and water balance, individual factors of unknown nature, technics of sweat collection as noted previously, and abnormality of sweat gland function (Darling *et al.*, 1953 ; di Sant'Agnese, 1956 ; Gochberg and Cooke, 1956). Characteristic differences in the salt concentration of sweat depending on sex and race have not been established ; reports to the contrary are based on observations made without control of diet or standardization of stimulus or collection methods. The frequently observed direct correlation of sweat sodium or chloride concentration with the rate of sweat secretion appears to be a secondary consequence of an usual, but not invariable, correlation between skin temperature and sweating rate, the primary relationship† being a direct one between the skin temperature, presumably reflecting the temperature of the sweat glands, and the sweat salt concentration (Robinson *et al.*, 1950b ; Weiner and van Heyningen, 1952a). The diminished ability of the glands at elevated temperatures to continue producing sweat of a low salt concentration has been regarded by some as evidence of glandular fatigue (Hancock *et al.*, 1929 ; Johnson *et al.*, 1944 ; Ladell, 1945 ; Robinson and Gerking, 1947), the glands being unable to maintain the osmotic gradient between the sweat and the extracellular fluid, but no suggestion is offered as to the nature of the particular secretory process or component which may be failing. The sweat salt concentration rises during the course of a single episode of sweating, quantitative discrepancies among various reports of this phenomenon depending on differences in experimental technique.

Kuno (1956) suggests that this rise in sweat salt concentration is due to a progressive accumulation of salt in the skin, since in the course of elaboration of a hypotonic secretion, the sweat glands remove relatively more water than chloride from the interstitial fluid surrounding them. As sweating continues, the glands receive materials for sweat production from a salt-enriched interstitial fluid, and consequently the sodium and chloride content of sweat gradually rises. The delay in reduction of the sweat salt concentration with subsidence of sweating is explained by the persistence and gradual decline of the elevated salt content of the skin. To the extent that there is no osmotic inactivation of sodium or chloride in the interstitial space, an increase in salt concentration of the interstitial fluid will accelerate the filtration of fluid from the capillaries, elevate the interstitial fluid pressure, and thus increase the rate of bulk delivery of material to the secreting cells. Evidence for salt accumulation in skin during sweating was obtained by Yoshimura (personal communication) who made direct measurements of the chloride ion activity with a special Ag,AgCl electrode inserted into the skin to a depth of 2 or 3 mm where the sweat gland tubules are most abundant.

Dill *et al.* (1933) were the first to point out that the salt concentration of sweat decreases during the course of acclimatization to heat. This phenomenon has been confirmed repeatedly (Bass *et al.*, 1955) and has been shown to depend on excessive loss of salt in the sweat with subsequent development of a negative salt balance (McCance, 1938 ; Conn, 1949a ; Robinson *et al.*, 1950a ; Weiner and van Heyningen, 1952b) and to be mediated by increased secretion of aldosterone (Collins *et al.*, 1955 ; Streeten *et al.*, 1955). The evidence for participation of pituitary

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† This relationship does not obtain in anoxia. Van Heyningen and Weiner (1952b) showed that the chloride concentration of sweat from an ischemic arm bore no relation to the temperature of the skin area from which the sweat was collected.

ACTH (Conn *et al.*, 1946 ; Conn, 1949a, b ; Conn and Louis, 1950) in this regulation has been reviewed recently by Bass *et al.* (1955), who regard it as inconclusive. The reduction in sweat salt concentration requires about a day to develop following establishment of an adequate salt deficit, although 3 or 4 days are required for reversal of the effect after salt replacement (Robinson *et al.*, 1950a). Robinson *et al.* (1950a) also observed a dissociation of the general metabolic and cardiovascular changes and the change in the salt concentration of the sweat during acclimatization, suggesting that the latter effect was incidental to the essential adaptive mechanism. More recently Robinson and co-workers (Robinson *et al.*, 1955, 1956 ; Pearcy *et al.*, 1956) have analyzed the discrepant reports concerning the interrelationship between the salt and water balance of the body and the excretion of salt and water by the sweat glands and kidney. The conflicting data in the literature are explained by the differences in time required for the kidneys and the sweat glands to respond to deficit, replacement, or excess of salt and/or water. Their experimental procedure enabled separate and simultaneous evaluation of the effects of salt deficiency and dehydration. Salt depletion evoked salt conservation by both the kidneys and sweat glands, the responses being less marked when the subjects were dehydrated than when water losses were replaced during the experiments. Renal effects were observed in the second and third hour of the experiments, whereas changes in the sweat chloride concentration were not clear until the 14th, and in some instances, the 24th hour. Thus, at a given time, the sweat salt concentration may represent a response to a previous state of salt deficit or excess, whereas the simultaneous salt concentration of the plasma or the rate of excretion of salt in the urine represents a response to more recent salt intake because the renal adjustment is so much more rapid.

The ratio of the concentration of sodium to the concentration of chloride in the sweat usually ranges from 1.0 to 1.3, although extremes of 0.7 and 2.0 have been observed. The Na/Cl ratio has been noted to fall during acclimatization to work in the heat (Dill *et al.*, 1933, 1938 ; Robinson *et al.*, 1950c). Also, the ratio has been noted to decrease following administration of deoxycorticosterone acetate (Conn, 1949b ; Conn and Louis, 1950) and during adaptation to salt deficiency (McCance, 1938), but this has not been a consistent finding (Moreira *et al.*, 1945 ; Leslie and Levin, 1950 ; Robinson *et al.*, 1950c ; Locke *et al.*, 1951).

Clinical interest has focused on the use of the salt concentration of the sweat as an index of the level of activity of adrenal cortical salt-conserving steroids (Conn, 1949b ; Conn and Louis, 1950 ; Locke *et al.*, 1951 ; Schwartz *et al.*, 1955a) and on the finding that the concentrations and rates of excretion in sweat of sodium and chloride are two to four times higher than normal in individuals with fibrocystic disease of the pancreas and also in some of their apparently unafflicted relatives (Darling *et al.*, 1953 ; Shwachman and Leubner, 1955 ; di Sant'Agnese, 1956 ; Gochberg and Cooke, 1956). The elevated sweat-salt content in the latter condition is due to abnormality of the sweat gland tubules per se, not to impaired renal or adrenal function, and in fact has proved to be a much more reproducible and reliable diagnostic index in patients with fibrocystic disease than it has in patients with adrenal cortical hypofunction. In the latter connection, it is to be hoped that there will be a continuation or renewal of attempts to control nonhormonal influences on sweat composition in order to improve sweat assays for aldosterone and its congeners, since the effects of these steroids on sweat sodium concentration, unlike their temporary effects on renal sodium reabsorption, are sustained as long as the steroids continue to be introduced into the circulation.

b. Potassium. Potassium appearing on the skin surface derives from superficial epidermal cells, sebum, and insensible, as well as visible, sweat. After a period of rest in a cool environment, twice as much potassium as sodium appears in the water washings of adult human skin (Hancock *et al.*, 1929). Kleeman *et al.* (1953) considered desquamation a minor source of nonsweat potassium and nitrogen because the phosphorus content of skin washings would be expected to be much higher than observed (*vide infra*) if there were a large cellular contribution of minerals. With the appearance of sweat secretion, the Na/K ratio increases from approximately 0.5 to levels of from 2 to 15. Thus sweat, which is minimally contaminated with salt from sebum and epidermal tissue, contains more sodium than potassium. The K concentration of thermal sweat is usually higher than the plasma K concentration and averages about 6 meq/liter with individual values ranging from 1 to 30 meq/liter (Rothman and Schaaf, 1929 ; Fishberg and Bierman, 1932 ; Dill *et al.*, 1933 ; Talbert *et al.*, 1933 a, b ; Hopf, 1935 ; Whitehouse, 1935 ; Darrow *et al.*, 1949 ; Ahlman *et al.*, 1952, 1953 ; Berenson and Burch, 1953 ; Kleeman *et al.*, 1953 ; Amatruda and Welt, 1953 ; Robinson and Robinson, 1954 a, b ; Gochberg and Cooke, 1956 ; Schwartz, Thorn, and Thaysen, unpublished data).

There are not enough data available to permit any clear statements of the relationship to the sweat K concentration and K excretion rate of such variables as the sweating rate, skin temperature, manner and nature of secretory stimulus, time during a single period of sweating, state of acclimatization, glandular fatique, anoxia, exercise, hormonal activity, dietary changes, and the state of the Na and K balance of the body. Such data as are available on these relationships must be considered preliminary. Kleeman and associates (1953) have shown that arm sweat collected under an impermeable vapor barrier had consistently higher concentrations of K and N but inconsistently higher concentrations of Na and Cl[†] than sweat allowed to evaporate freely from the total body surface. Several investigators (Hancock *et al.*, 1929 ; Whitehouse, 1931 ; Kleeman *et al.*, 1953 ; Bass *et al.*, 1955 ; Berenson and Burch, 1953 ; Schwartz and Thaysen, 1956) have observed that immediately following stimulation of the glands, sweat is secreted with a higher concentration of K (and N)[‡] than in subsequent samples, in which the K concentration has been found to fall promptly to variably lower and usually constant levels until very low rates of flow are reached, when the K concentration again rises. This latter phenomenon, frequently observed in the case of the secretion of saliva (Langstroth *et al.*, 1938 ; Thaysen *et al.*, 1954 ; Hildes, 1955 ; Thorn *et al.*, 1956), has been analyzed in exquisite detail by Burgen (1956), who found the concentration of K in saliva secreted from a previously rested gland to have an initial transient phase which passes into a lower steady-state level. During the transient phase the gland cells lose K into the blood as well as the saliva and undergo a partial replacement of intracellular K with Na. Thereafter, the output of K from the gland is balanced by equivalent extraction from the blood. The early washout of K and replacement with Na may be primarily implicated in the activation of secretion or may simply be a gratuitous secondary consequence of diversion of energy sources channeled to maintain normal resting ionic gradients to processes concerned with initiating and establishing a steady state of secretory activity (Schwartz *et al.*, 1958).

In several studies the K concentration in sweat has been found to vary inversely with Na concentration (Locke *et al.*, 1951 ; Schwartz *et al.*, 1955a ; Schwartz and Thaysen, 1956, Schwartz and McMaster, unpublished data), when the latter was varied by the direct administration of salt-conserving adrenal steroids or indirect manipulations of the sodium and potassium intake and balance. In circumstances causing reciprocal variations of the sweat Na and K, the Na/K ratio obviously is a better quantitative index of effect than the changes in concentration of either ion alone. Thus, increases in the sweat K concentration, but more striking decreases in the sweat Na/K ratio, have been observed following administration of adrenal cortical extract (Moreira *et al.*, 1945 ; Schwartz and Thaysen, unpublished data), DCA and ACTH (Conn, *et*

[†] The lower the concentrations of Na and Cl in arm bag sweat, the more closely they approximated the concentrations of Na and Cl in total body sweat.

[‡] Kleeman *et al.* (1953) considered these higher levels of K and N to be due either to concentration of stagnant sweat remaining in the quiescent sweat gland lumen or to sebaceous contamination and have emphasized the importance of a preliminary " flushing " period in order to obtain meaningful or " true " values for the concentration of K and N in unmodified sweat.

al., 1947; Conn, 1949a, b; Conn and Louis, 1950), and aldosterone (Schwartz and McMaster, unpublished data), and during adaptation to salt deficit produced by intent and, doubtless, by incident during acclimatization to heat (Dill *et al.*, 1933; Ahlman *et al.*, 1952). Recently Bass *et al.* (1955) found a fall in the sweat K as well as Na concentration in the course of acclimatization to heat. Since their subjects were on high salt intakes and in sodium balance and showed no change or an increase in the sweat Na/K ratio, it appears that the characteristic decrease in sweat Na and Cl observed during acclimatization to heat is not simply or, at least, exclusively dependent on the development of salt deficiency, nor, therefore, on enhanced adrenocortical secretion of salt-conserving steroids.

c. Calcium. The sweat calcium concentrations that have been reported in various studies range from 0.3 to 12.2 mg % (Marchionini and Ottenstein, 1931; Borchardt, 1931; Bryant and Talbert, 1931; Carpenter and Talbert, 1932; Fishberg and Bierman, 1932; Talbert *et al.*, 1933a; Hopf, 1935; Mitchell *et al.*, 1949; Johnston *et al.*, 1950). The concentration in sweat is almost always lower than the concentration in whole plasma and usually lower than the concentration in the plasma fraction that is dialyzable through common cellophane membranes. The sweat calcium concentration varies inversely with the secretory rate; it is high in the initial sweat samples but falls progressively during a single sweating period (Hopf, 1935; Mitchell *et al.*, 1949). Under comfortable conditions, with sweating at a minimum, Mitchell *et al.* (1949) found an average dermal calcium excretion of 6.2 mg/hr or 149 mg/day; thus there appears to be considerable loss with epithelial debris and/or sebaceous secretion. When the same individuals, all young men, sweated profusely in a hot humid environment, the average cutaneous calcium loss increased to 20.2 mg/hr and amounted to 30 % of the total calcium loss from the body.[†] The fecal loss increased by approximately the same amount, but the urinary loss did not change. Although the dermal and fecal increments were similar in magnitude and thus contributed approximately equally to the unfavorable change in total calcium balance, the former represented an increase of over 225 % and the latter of only 44 % when calcium losses under comfortable "nonsweating" conditions were compared with losses in a hot humid environment. The foregoing studies do not constitute by any means an adequate survey of possible compensatory relationships between losses through the three major excretory pathways: skin, kidney, and alimentary tract. Further work is needed either to exclude firmly or to uncover the nature and extent of such compensatory relationships before information about the dermal pathway alone can be utilized properly to appraise nutritional requirements and to guide the planning of metabolic balance experiments.

[†] The calcium intake of these subjects averaged 856 mg daily when they were kept at comfort and 1144 mg daily when they were kept in humid heat, owing to a greater consumption of tap water under the latter circumstances. This difference in intake not only cannot account for the increased dermal calcium loss in the heat but suggests that the dermal loss would have been even greater if the calcium intake had been held constant, since an anomalous fall in both the rate of excretion and the concentration of calcium in the sweat occurs as a result of simple increase in the dietary calcium intake (Mitchell *et al.*, 1949).

In a somewhat similar study in which young women served as subjects, Johnston *et al.* (1950) found approximately the same values for sweat calcium concentration, but a much lower average rate of sweat production and a correspondingly lower average rate of calcium loss, viz., 8.54 mg per hour.

d. Magnesium. The magnesium concentration of sweat has been reported to vary from 0.04 to 4.5 mg % (Talbert *et al.*, 1933a; Hopf, 1935; Mitchell *et al.*, 1949). There is no consistent relation between the concentrations of Ca and Mg in the sweat, although the concentrations of both cations are highest when secretion starts and later decrease to lower values (Mitchell *et al.*, 1949). Either Mg, Ca, or both may occasionally be present in sweat in concentrations too low to be detected by the usual analytic methods.

e. Iron. The skin is an avenue for the excretion of iron chiefly by virtue of the high iron content of exfoliated epidermal cells (Rothman and Schaaf, 1929). The iron content of unfiltered opalescent cell-rich sweat ranges from 0.1 to 2.0 mg % (Adams *et al.*, 1950), whereas the filtrate or supernatant after centrifugation has an iron content ranging from 0 to 0.5 mg % (Mitchell *et al.*, 1949; Adams *et al.*, 1950; Johnston *et al.*, 1950; Dubach *et al.*, 1955). It is possible that apocrine sweat contributes to the dermal excretion of iron since there is a demonstrable iron deposit in the apocrine sweat gland (Homma, 1926; Bunting *et al.*, 1948). Iron has not been demonstrated in the cells of eccrine glands except in hemochromatosis (Ungeheuer, 1914).

The quantitative importance of cutaneous iron loss is a disputed issue. Mitchell *et al.* (1949) reported that the rate of dermal excretion in 4 young men ranged from 0.134 to 0.528 mg per hour, averaging 0.27 mg hourly (6.5 mg daily) under environmental conditions which evoked minimal sweating. Dermal excretion was increased approximately fivefold to an average value of 1.29 mg of iron per hour when sweating was initiated by bringing the subjects into a hot humid environment. Under the latter circumstances the dermal iron loss was 6 times greater than the renal output and amounted to 13.4 % of the total iron excretion from skin, kidneys, and alimentary tract. With minimal sweating, the dermal loss was 37.2 % of the total iron excretion, and over 50 times as great as the renal output. There was no evidence that the iron content of the sweat was affected by changes in either the sweating rate or the oral intake of iron; nor was there more than a faint suggestion of compensatory variation in renal, alimentary, and dermal iron losses.

In 4 young women exposed to humid heat Johnston *et al.* (1950) found that iron was lost through the skin at only 6% of the rate and 12% of the concentration reported by Mitchell *et al.* (1949). In attempting to account for the discrepancy, Johnston *et al.* (1950) pointed out: (1) that the sweating rates of the men in Mitchell's study were more than twice those of the women in her group; (2) that, since the apocrine glands are associated with hair follicles, the production of presumably iron-rich apocrine sweat should be higher in men than women because of the higher ratio of apocrine to eccrine output as well as the higher rate of total sweat flow; and (3) that her collections, unlike Mitchell's, did not include scalp sweat which has a high apocrine component.

Differences in chemical analytic methods fail to explain the discordant findings, which possibly are the result of variations under the different experimental conditions of contamination from epidermal debris,† if not from external sources, and of the rate of secretion of other sweat components, for example: (1) hydrogen ions, since low pH increases iron solubility, and high pH (values about 7) decreases iron solubility by favoring formation of colloidal ferric hydroxide or ferric hydroxide phosphate which then adsorbs on to cell debris; (2) presence of substances such as ascorbic acid which solubilize iron by reducing it from the ferric to the ferrous form; (3) presence of substances such as citrate or lactate which form soluble ferrous complexes and retard autoxidation of iron; (4) presence of oxygen and phosphate which promote autoxidation; and (5) presence of protein or other substances which may chelate or loosely bind iron.

After intravenous administration of radioactive iron isotopes, Fe^{59} and Fe^{55}, to normal humans and patients with various anemias, the total iron loss was reported to vary from 0.33 to 0.52 mg per day in normal subjects and in an individual with hypoplastic anemia, from 0.03 to 0.06 mg per day in iron-deficient subjects, and from 0.3 to 1.45 in two patients with hemolytic anemia; only traces of the injected dose of isotope were excreted through the skin (Dubach *et al.*, 1955). Iron losses were calculated from measurements of the excreted radioactivity on the assumption that the isotope had distributed itself uniformly throughout the total body iron. If, however, such uniform distribution had not been attained at the time of sampling, and if peripheral iron deposits, as in epidermal cells and hair, had a lower specific activity than assumed, the calculated dermal, and therefore, total iron losses would have been underestimated. Therefore, it is possible that a very low rate of isotope exchange or incorporation in peripheral iron compartments may account also for the failure of Stewart *et al.* (1950) to find significant amounts of radioactivity in the skin washings (which presumably contained desquamated cells) or the hair of dogs previously given Fe^{55} intravenously or by stomach tube. However, even if appreciable dermal losses of iron ensue under special conditions favoring desquamation, increased sweat secretion, and/or solubilization of iron in sweat, a low rate of epidermal cell iron turnover would preclude the possibility that the skin has an important reservoir function in iron homeostasis.

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† It is possible that the final sample of sweat obtained in Mitchell's experiments had more iron-containing particulate matter either because of differences in fragmentation of desquamated cells or differences in filtering techniques.

It is perhaps worthy of comment that the small amount of iron excreted in saliva (Dubach *et al.*, 1955) is destined to pass through the alimentary tract largely unresorbed, like the iron ingested with food, excreted in bile, and contained in desquamated mucosal cells, but unlike other salivary constituents. The very limited gastrointestinal absorption of iron (Moore and Dubach, 1951) places importance upon even the smallest estimates of iron loss through the gut, kidney, and skin.

f. Copper and Manganese. Mitchell *et al.* (1949) found copper and manganese present in sweat in trace amounts. The copper concentration averaged 5.8 μg/100 ml with a range from 4.4 to 8.0 μg/100 ml; the manganese concentration averaged 6.0 μg/100 ml with a range from 3.2 to 7.4 μg/100 ml.

g. Bismuth, Mercury and Silver. Bismuth did not appear in the sweat of patients receiving large doses of the metal as antiluetic therapy (Schaffer, 1930), but according to Kuno (1956), mercury and silver can be discharged in sweat. In argyria, Tobioka (cited by Kuno, 1956) found silver deposited abundantly in the eccrine glands and less densely and less regularly around hair follicles and sebaceous glands.

h. Lead. In a recent study of 6 patients with industrial lead poisoning, and of one subject who ingested lead, Shiels (1954) has observed dermal excretion of from 0.002 to 0.046 mg of lead from an area of axillary skin of approximately 50 square centimeters. Although the method of sweat collection used did not permit measurement of the amount of lead actually secreted in the sweat, as distinguished, for example, from lead lost with desquamated epithelial elements, it is clear that, either directly or indirectly, an increase in the sweating rate is associated with an increased elimination of lead in the sweat. Also, it appears that the skin may be a much more significant excretory route than the kidney, a factor of significance in the decreased incidence of lead intoxication associated with the hot summer climate (Shiels, 1955).

i. Fluorine, Bromine, and Iodine. The concentration in sweat and the rate of dermal excretion of fluorine (Machle *et al.*, 1942 ; McClure *et al.*, 1945), iodine (Spector *et al.*, 1945 ; Nelson *et al.*, 1947) and bromine (Cornbleet, 1938) all rise after increased intake and absorption. As the sweating rate rises the dermal fluorine loss also rises moderately, although the sweat concentration falls. Balance is usually maintained when the daily fluorine intake does not exceed 4–5 mg. This is accomplished by excretion chiefly via the kidneys and skin.

Bromine is normally present in sweat and exfoliated cells. The normal concentration has been observed by Cornbleet (1938) to range from 18.2 to 50.2 μg % in unfiltered sweat and from 16.5 to 41.2 μg % in filtered sweat. Following bromide ingestion the cellular bromide content increases markedly, but the sweat concentration rises only from 25 to 50 % above control levels, a moderate elevation which is maintained until the abnormal bromide accumulation of the body has been removed.

In 3 normal young men Spector *et al.* (1945) found an iodine concentration of approximately 1 μg % in undiluted, and presumably unfiltered, sweat. After a single dose of 2 mg of potassium iodide the concentration rose to approximately 3 to 4 μg %. Profuse sweating had no striking or consistent effect on dermal iodine excretion at low levels of intake (700 μg per day) but increased dermal losses on higher intakes (2200 μg per day). At all levels of intake, more than 75 % of the iodine lost from the body was excreted in the urine, and the sweat/plasma iodine concentration ratio was approximately 0.35 and paralleled the sweat/plasma chloride concentration ratio. Fecal iodine excretion is negligible (Spector *et al.*, 1945 ; Nelson *et al.*, 1947).

j. Phosphorus and Sulfur. Talbert *et al.* (1933b) found the total phosphorus content of sweat to range from 1.55 to 3.34 mg/100 ml, the higher values being associated with a high phosphorus diet and/or with sweat secreted during work rather than as the result of mere exposure to heat. Other workers have found little or no phosphorus in sweat, reporting maximal concentrations of 0.04 mg/100 ml (Bischoff *et al.*, 1931 ; Whitehouse, 1935 ; Mitchell *et al.*, 1949). Alkaline phosphatase has been localized in the sweat-gland cell membranes and nuclei, but the function of " high energy " and other organic phosphorus compounds in the secretory process remains to be defined.

The total sulfate content of sweat has been reported to vary from 0.7 to 19 mg/100 ml of sweat (Talbert *et al.*, 1933b ; Mosher, 1933 ; Whitehouse, 1935). Inorganic sulfate amounted to 50 % or less than the total sulfate determined on ashed aliquots of sweat. High values for sulfate as such and for ash sulfate, observed at low sweating rates and particularly in the initial stage of sweating, were ascribed to epidermal contamination (Whitehouse, 1935).

k. Total Nitrogen. An average adult under comfortable conditions sustains a dermal loss of approximately 0.3 ±0.1 gm/day of nitrogen under temperate climatic conditions without visible sweating (Bost and Borgstrom, 1926 ; Rothman and Schaaf, 1929 ; Peters, 1935 ; Mitchell *et al.*, 1949). It is difficult to estimate precisely how much of the nitrogen loss is due to shed epidermal elements and how much of it may be attributed to the diffusion and the secretory components of insensible perspiration, but Voit (1930) found that about one-third of the skin nitrogen loss represented keratin nitrogen and the remaining two-thirds was ascribed to "insensible sweating." Voit also reported the daily nitrogen loss from hair and nail growth to be 24 mg ; thus the total nitrogen losses attributed to keratinized material were estimated to be approximately 100 mg per day for average normal young adults.

The total nitrogen content of sweat has been reported to vary from 17 to 196 mg % (Robinson and Robinson, 1954a ; Rothman, 1954 ; Kuno, 1956). The total output of nitrogen in sweat may exceed 5 gm/day under conditions of climate and activity leading to sustained high sweating rates and elevated levels of blood nonprotein nitrogen concentration. Mitchell *et al.* (1949) found the output of nitrogen in sweat to increase from 16 mg/hr under comfortable conditions to 180 mg/hr under hot humid conditions but pointed out that this increase in nitrogen output in the sweat does not imply a corresponding increase in protein requirement since so little is known about possible compensatory variations in the urinary nitrogen output (Mitchell and Edman, 1951).

l. Ammonia Nitrogen. Ammonia nitrogen was found to constitute 3–6.5 % of the total nitrogen of the water washings of normal, dry, nonsweating skin (Rothman *et al.*, 1949). The concentration of ammonia nitrogen in sweat has been reported to range from 1 to 35 mg % (Talbert *et al.*, 1927 ; Mosher, 1933 ; McSwiney, 1934 ; Robinson *et al.*, 1941 ; Lobitz and Mason, 1948a ; Itoh and Nakayama, 1953 ; Amatruda and Welt, 1953 ; Kleeman *et al.*, 1955) and to exceed the plasma concentration by 25- to 250-fold. Much of this scatter arises undoubtedly from variable degrees of contamination owing to the action of bacterial ureases and possibly other ammonia-generating enzymes in the epidermis. In one study (Gad-Andresen, 1921) the concentration of ammonia in the sweat was observed to parallel the concentration of ammonia in the plasma, but no such correlation was found in more recent studies with precautions to avoid secondary decomposition of urea in stagnant sweat (Amatruda and Welt, 1953 ; Kleeman *et al.*, 1955). Such contamination may be responsible for the very much higher ammonia concentration in axillary as compared with general body sweat, especially in view of the finding of a lower concentration of urea in axillary sweat as compared with general body sweat (Ottenstein, 1950 ; Thurmon and Ottenstein, 1952). During a given period of sweating, the ammonia concentration was found to be high in the initial samples, but within 15 to 30 minutes it fell and remained at a constant level despite subsequent changes in the rate of sweat secretion (Itoh and Nakayama, 1953).

Considerable ammonia production was noted in human skin slices in the presence of glutamine, but not of glycine, alanine, glutamic acid, threonine, asparagine, tyrosine, histidine, and urea, suggesting that glutamine is the predominant precursor of the ammonia of sweat. Under these circumstances the formation of ammonia from glutamine, in the presence of phosphate but not in the presence of pyruvate, showed the glutamine-deaminating enzyme of human skin to be glutaminase I (Itoh and Nakayama, 1953). No glutaminase activity was found in sweat. During acidosis produced by ammonium chloride ingestion, Amatruda and Welt (1953) found a rough inverse correlation between the concentrations of ammonia and sodium in the sweat, although, as they pointed out, the increase in ammonia concentration was small, whereas the decrease in sodium concentration was much greater. Furthermore, their data and those of Kleeman and associates (1955) do not support the suggestion (Kuno, 1956) that during acidosis increased ammonia production by the sweat glands plays a significant role in the conservation of fixed base.

m. Other Nitrogenous Constituents of Sweat. The concentration of amino acid nitrogen in sweat varies in the range of 1 to 10 mg %, exceeding the plasma concentration at low sweating rates and falling to or below the plasma level at intermediate and high sweating rates (Mosher, 1933; McSwiney, 1934; Itoh and Nakayama, 1953; Kuno, 1956). Thus, the loss of amino acids in sweat even under conditions of heat and work does not seriously affect the nitrogen economy of the body. One-half of the nitrogen in water washings of the normal nonsweating skin surface proved to be amino nitrogen (Rothman *et al.*, 1949). Using paper chromatography Rothman and Sullivan (1949) found twelve individual amino acids (aspartic and glutamic acids, serine, glycine, alanine, citrulline, arginine, hydroxyproline, lysine, valine, leucine, tyrosine) and hydroxylysine, threonine, lysine, and ornithine were identified tentatively. In a similar experiment on a patient with anidrotic ectodermal dysplasia, Rothman and Sullivan (1949) found only traces of serine and alanine, thus indicating that the amino acids of the skin surface had been derived from sweat whether visible or not. By microbiological assay, Hier *et al.* (1946) identified valine, leucine, isoleucine, lysine, phenylalanine, tryptophan, arginine, histidine, threonine, and tyrosine in sweat, with the latter four amino acids at variably higher concentrations than in the blood. With paper chromatography, Itoh and Nakayama (1952) observed the presence in sweat of the following additional amino acids ; serine,† glutamic acid, aspartic acid, proline, methionine, and cystine. Araki and Ando (1953) did not find isoleucine, tryptophan, nor proline in sweat but otherwise confirmed the earlier observations. Glycine, alanine, and glutamine, although present in relatively high concentrations in the skin and plasma, have not been found in the sweat. By and large, variations in blood levels produced by feeding large amounts of amino acids have had little or no effect on their concentrations in sweat (Hier *et al.*, 1946), indicating that in general amino acids do not simply diffuse into the sweat from the interstitial fluid.

The concentration of urea nitrogen in sweat has been studied by a number of investigators who have reported variation from 5 to 150 mg % (Schwartz *et al.*, 1953 ; Robinson and Robinson, 1954a). In general, the concentration of urea in the sweat, S, is twice the concentration of urea in the plasma, P, the ratio S/P remaining unchanged despite more than 50-fold variation in P. The S/P ratio is also independent of the sweating rate except at the earliest stage of sweating and at very low rates of secretion when it may be as high as 20. Variations in urea concentration in the sweat have been observed also to depend on the area of sampling and the technique of sweat collection (van Heyningen, 1951 ; Kleeman *et al.*, 1953). The finding that the urea was consistently more concentrated in sweat than in blood was interpreted as evidence that the sweat glands initially produce a primary secretion which is subsequently modified by the reabsorption of water (Schwartz *et al.*, 1953). Rothman *et al.* (1949) showed that urea is deposited in substantial amounts on the skin surface in the absence of visible sweating.

Creatinine contributes only 1–2 % of the total nitrogen found in water washings of normal nonsweating human skin (Rothman *et al.*, 1949). The concentration in sweat of creatinine as determined by the classical Jaffe reaction has been reported to vary from 0 to 6.7 mg % (Rothman and Schaaf, 1929 ; Mosher, 1933 ; Mickelson and Keys, 1943 ; Ladell, 1947 ; Lobitz and Mason, 1948b ; Bass and Dobalian, 1953). There is a roughly inverse relation between the sweating rate and the concentration of Jaffe-reacting chromogen in the sweat (Schumann, 1931 ; Ladell, 1947), the latter falling and then later rising towards initial levels during an exposure to heat (Ladell, 1947). Bass and Dobalian (1953) have shown that only 29 ± 7 % of the Jaffe-reacting chromogen in sweat can be considered to be " true " creatinine, as determined by Hare's (1950) modification of the method of Borsook (1935), which involves adsorption on Lloyd's reagent and subsequent elution with alkaline picrate. Creatine as well as creatinine has also been reported in some samples of sweat (Schumann, 1931).

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† Serine was the first amino acid to be identified in sweat (Embden and Tachau, 1910).

Uric acid was not detected in water washings of 2 square meters of normal nonsweating skin (Rothman *et al.*, 1949). The concentration of uric acid in thermal sweat has been found to range from 0 to 1.7 mg % and is always lower than the concentration in the blood (Lobitz and Mason, 1948c). Adler (1916) reported an elevation of the sweat uric acid concentration to 30 mg % in a subject on a high dietary intake of nucleoprotein; and Lobitz and Mason (1948c) observed moderate increases in the uric acid concentration of palmar sweat in proportion to increases in blood level following intravenous injection of lithium urate.

n. Other Organic Constituents. A limited discussion of some other organic constituents of eccrine sweat is included not only to complete our general consideration of the skin as an excretory pathway but because of the important, although undefined, relation of the metabolic activity of the sweat gland to the transport of water, mineral, and other sweat components. In the latter connection, there has been interest in the excretion in sweat of reducing substance (as an approximation for glucose), pyruvic acid, and particularly lactic acid. The concentration of reducing substance in sweat ranges from 0 to 11 mg %, averaging 3 mg %, and responds minimally but in a parallel direction to change in the blood glucose concentration. When measured specifically as the phenylglucosazone, the true glucose present in the sweat and on the skin surface was found to be only about one-tenth of that estimated from measurement of the total reducing substance (Schulze and Kunz, 1940). The nature of the bulk of the reducing substances excreted by the skin remains unknown.

The lactic acid content of sweat may derive in part from the extracellular fluid as was once believed, but studies during recent years indicate that its major source is the anaerobic metabolism of glycogen and glucose by the sweat glands. The sweat lactate concentration ranges from 4 to 40 meq/liter or 4–40 times the plasma lactate concentration, of which it appears to be independent (Dill *et al.*, 1933; Whitehouse, 1935; Weiner and van Heyningen, 1952c). It increases when the glands are rendered anoxemic after arterial occlusion (van Heyningen, 1951; Weiner and van Heyningen, 1952c) and during the initial stage of sweating, presumably before anoxemic cutaneous vasodilation has taken place (Whitehouse, 1935; Itoh *et al.*, 1952). At the lowest concentration of lactate found, its anaerobic derivation from glucose suffices by calculation to provide all of the energy required to produce the most dilute sweat specimen observed. However, despite the ample and readily mobilizable glycogen deposit in the sweat gland (Bunting *et al.*, 1948) and despite the theoretical possibility that anaerobic glycolysis may provide the energy for sweat formation, large changes in the osmotic work of the glands generally have not been well correlated with changes in the concentration and rate of secretion of lactate in sweat (Weiner and van Heyningen, 1952c).

The concentration of pyruvic acid in the sweat ranges from 0.1 to 0.8 meq/liter and may be 10 times the plasma pyruvic acid concentration. Like the concentration of reducing substance and lactic acid, the pyruvate concentration is highest in the initial stage of sweating and falls with subsequent increase in the sweating rate, findings which have been attributed in part to an increased utilization of sugar and its intermediates as sweating continues, and in part to an initial washout of accumulated epidermal pyruvate and lactate (Itoh *et al.*, 1952).

Thiamine, riboflavin, nicotinic acid and its metabolites, pyridoxine and its metabolites, pantothenic acid, choline, inositol, *p*-aminobenzoic acid, folic acid and F_1 and F_2 of Najjar and Holt, dehydroascorbic acid and occasionally ascorbic acid have been identified in sweat (Mickelsen and Keys, 1943; Sargent *et al.*, 1944; Mitchell and Edman, 1951; Robinson and Robinson, 1954a). Selection of the most reliable data as judged from appraisal of the method of assay, technique of sweat collection, and agreement with other findings showed that the water-soluble vitamins are excreted in the sweat in amounts that are too small to affect nutritional requirements (Mitchell and Edman, 1951). Except for dehydroascorbic acid, pantothenic acid, and possibly pyridoxine, the vitamin concentration of sweat was not observed to be related to the dietary intake.

Other substances that have been recognized in the sweat include acetylcholine, alcohol, citric acid, histamine, phenol, indican, adrenal corticoids, diphtheria antitoxin, atabrine, morphine, nicotine, and sulfonamides.

o. pH. The acid reaction of the aqueous film on the human skin surface (average pH 4.5, range 3-7) has been observed repeatedly since the first direct measurements with a quinhydrone electrode (Rothman, 1954; Schirren, 1955). This acidity is presumed to be due to the deposit and evaporation of thermal eccrine sweat, the pH of which has been found usually in the range of 4 to 6.8 (Robinson and Robinson, 1954a; Herrmann and Mandol, 1955; Kleeman *et al.*, 1955). In general it has been observed that the pH of sweat rises with time as sweating proceeds continuously (Kittsteiner, 1910-1911; Talbert 1920; Adolph, 1923; Karitzky *et al.*, 1949) or intermittently (Hardt and Palmer, 1937), although Dill *et al.* (1938) noted that the pH of successive samples of hand sweat fell progressively in one subject but rose in another. Shelley and Hurley (1953) have found that apocrine sweat was only slightly more alkaline than eccrine sweat, despite earlier reports of a greater disparity (Marchionini and Hausknecht, 1938; Thurmon and Ottenstein, 1952).

The mode of stimulation of the glands has considerable influence on the pH of the sweat produced, the highest pH values having been observed after intense local stimulation (Herrmann and Mandol, 1955). Amatruda and Welt (1953) and Kleeman *et al.* (1955) failed to observe a significant or consistent change in the pH, titratable acid, sodium, potassium, chloride, ammonia, or lactic acid concentrations of sweat in response to alterations of the acid-base balance of the body with ammonium chloride, sodium bicarbonate, or 6063, a potent carbonic anhydrase inhibitor. Thus, the sweat glands do not appear to play a role in the regulation of the reaction of the body fluids, despite an earlier suggestion to the contrary (Hopf, 1935).

Whether or not the transport of lactic acid is the unique or primary process that determines the sweat pH, the lactic acid/lactate conjugate pair is the predominant buffer system in the sweat. Its dissociation constant is 1.38×10^{-4}, and thus it has its maximum buffering capacity in the observed range of sweat pH. The dissociation constant of the carbonic acid/bicarbonate system is out of the effective buffering range, and the negative effect of 6063 on sweat composition excludes a significant role for carbonic anhydrase in the regulation of the sweat pH. Dicarboxylic acids have been observed on the skin surface (Rothman and Sullivan, 1949) but not in the sweat, although Hier *et al.* (1946) found glutamic and aspartic acid in sweat in greater concentration than basic amino acids. There is no evidence that water-soluble fatty acids (propionic, butyric, pentanoic, hexanoic) are present in sufficient concentration on the skin surface or in the sweat to contribute significantly to the acidity of either (Blank, 1939a, b ; Lincke, 1949).

Whatever the nature of the processes responsible for bringing about and maintaining the acidity of sweat, the gland cells are relatively freely permeable to neutral molecules and impermeable to ions (Thaysen and Schwartz, 1953), and therefore a pH gradient may be expected to result in an unequal distribution of weak electrolytes across the sweat gland tubule as has been demonstrated in the case of the renal tubule (Orloff and Berliner, 1954, 1956 ; cf. Vol. 1, Chapter 9). Thus weak acids, diffusing through the epithelium as neutral molecules, accumulate in the relatively alkaline extracellular fluid where they are trapped as nondiffusible anions ; whereas weak bases gain protons and are trapped as cations in the acid sweat. The precise partition of a weak electrolyte between the extracellular fluid and the forming sweat will depend not only on the pH but on the exact composition of the two fluids, on the rapidity with which the neutral molecules reach equilibrium, and on whether or not the impermeability to the ionic species is partial or complete.

The partition of a weak acid, base, or ampholyte between two aqueous compartments A and B, separated by a barrier impermeable to ions but permeable to neutral molecules, can be formulated for the special case in which the latter reach equilibrium. The mass action law of ionization is used to compute the distribution of electrolyte between A and B in terms of its thermodynamic dissociation constant K and the pH in A and B when the barrier between A and B is permeable to the weak acid HX but not to its ionic constituents, X^- and H^+. The total amount T_A of the weak acid in compartment A is $(HX)_A+(X^-)_A$; the total amount T_B in B is $(HX)_B+(X^-)_B$, the parentheses indicating that activities are being considered. T_A/T_B equals R, the partition ratio of the total acid between A and B. At equilibrium, the activities of the neutral molecular species in both compartments are equal:

$$(HX)_A=(HX)_B=(HX).$$

From the mass action law of ionization of a weak acid we have:

$$\frac{(X^-)_A(H^+)_A}{(HX)_A}=K=\frac{(X^-)_B(H^+)_B}{(HX)_B} \tag{1}$$

At equilibrium, therefore,

$$(X^-)_A(H^+)_A=(X^-)_B(H^+)_B \tag{2}$$

By definition,

$$R=\frac{(HX)+(X^-)_A}{(HX)+(X^-)_B} \tag{3}$$

and by rearrangement of Eq. (1),

$$(HX)=\frac{(X^-)_B(H^+)_B}{K} \tag{4}$$

Combining Eqs. (2), (3), and (4), we may solve for R, the partition coefficient of the acid between compartments A and B:

$$R=\frac{(H^+)_B[K+(H^+)_A]}{(H^+)_A[K+(H^+)_B]} \tag{5}$$

Rearranging, we may solve for $(H^+)_A$, the hydrogen ion activity in compartment A:

$$(H^+)_A=\frac{K(H^+)_B}{RK+(R-1)(H^+)_B} \tag{6}$$

which, in logarithmic form, may be written

$$\mathrm{pH}_A = \mathrm{p}K + \log[R(10^{\mathrm{pH}_B - \mathrm{p}K}) + (R-1)] \tag{7}$$

In the case of a dibasic acid at equilibrium in a two-compartment aqueous system, the partition ratio becomes :

$$R = \frac{(\mathrm{H}^+)_B[K_1(\mathrm{H}^+)_A + (\mathrm{H}^+)_A^2 + K_1K_2]}{(\mathrm{H}^+)_A[K_1(\mathrm{H}^+)_B + (\mathrm{H}^+)_B^2 + K_1K_2]} \tag{8}$$

and the hydrogen ion activity in compartment A,

$$(\mathrm{H}^+)_A = \frac{\begin{array}{c} RK_1(\mathrm{H}^+)_B + R(\mathrm{H}^+)_B^2 + RK_1K_2 - (\mathrm{H}^+)_BK_1 \\ \pm\{[RK_1(\mathrm{H}^+)_B + R(\mathrm{H}^+)_B^2 + RK_1K_2 - (\mathrm{H}^+)_BK_1]^2 - 4(\mathrm{H}^+)_B^2K_1K_2\}^{\frac{1}{2}} \end{array}}{2(\mathrm{H}^+)_B} \tag{9}$$

The latter equation has been used to calculate the hydrogen ion activity of human forearm sweat. The ampholyte, *p*-aminohippuric acid, the cationic form of which can be treated as a dibasic acid, was infused intravenously at a constant rate until uniform distribution was attained throughout the extracellular fluid, after which samples of sweat and plasma were obtained and analyzed for total *p*-aminohippuric acid concentrations.† The hydrogen ion activity of sweat, $(\mathrm{H}^+)_A$, was computed by substituting in Eq. (9) values of R and $(\mathrm{H}^+)_B$, the experimentally determined sweat/plasma *p*-aminohippurate concentration ratio and the plasma hydrogen ion activity, respectively. The values of $(\mathrm{H}^+)_A$, so calculated, correspond within 0.1 pH unit to the directly measured values for the hydrogen ion activity of the sweat (Schwartz *et al.*, 1955b). Thus, the distribution of *p*-aminohippuric acid across the sweat gland tubules conformed with the calculation based on the assumption of free permeability to the undissociated molecules but impermeability to the ions. It is of interest that this compound, which appears to be excreted in the sweat by diffusion, is the most studied, if not the most useful, example of a substance that is actively secreted by the kidney tubules.

The foregoing analysis of the partition of weak acids is of course applicable to weak bases and can be extended to the special cases of partial permeability to the ionic species, or partial diffusion hindrance to the neutral molecules, or combinations of both. Previous analyses of weak electrolyte accumulation and transfer in biological systems have been given by Osterhout (1925), Jacobs (1940), Danielli (1952), and Orloff and Berliner (1954, 1956).

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† No activity coefficient corrections were made for the *p*-aminohippurate concentrations.

5. *Discrete Processes and Mechanism of Secretion*

The findings that the concentration of urea in sweat was about twice that in plasma and that the mechanism transferring urea from extracellular fluid to sweat failed to exhibit saturation despite a 200-fold increase in the rate of excretion (Schwartz *et al.*, 1953) suggest that urea is concentrated in the sweat by reabsorption of water from a precursor solution, and not by specific secretion. If this is correct, the precursor must be concentrated by the reabsorption of a constant fraction of water as the urea concentration in the sweat is independent of the sweating rate. Unlike the kidney, where urine flow changes because of variable reabsorption of water from a relatively constant filtrate, the sweat gland appears to change flow by variation in the rate of formation of its precursor or primary secretion. In a later study (Schwartz and Thaysen, 1956) the manner of increase in sweat sodium concentration and the unchanging sweat potassium concentration with a rising rate of sweat secretion suggested that both ions are delivered into a precursor fluid in amounts proportional to the rate of secretion and that sodium, but not potassium, is reabsorbed from this precursor by a subsequent process of limited capacity. An alternative explanation for the increase in sweat sodium concentration with secretory rate lies in a possible action of acetylcholine on the transfer of sodium into the sweat gland cells and then into the forming sweat, higher concentrations of acetylcholine causing greater entry of sodium relative to water into the precursor.† Some direct evidence against the latter explanation was obtained in experiments in which acetylcholine and acetyl-β-methylcholine were infused intracutaneously at a constant rate. During such an infusion, the sodium concentration varied in parallel with the sweating rate which reached an early maximum and then declined exponentially with a half-time of approximately 15 minutes, just as after a single injection of mecholyl. The fall in both sodium concentration and sweating rate thus took place at a time when the delivery of mecholyl to the secreting site was either replenished continually or increased.

Lloyd (1957, 1959) has shown that during repetitive stimulation of the secretomotor fibers to the sweat gland of the cat's paw, the time required for visible emergence of sweat is proportional to the duration of the rest interval between the immediate and the preceding episodes of stimulation. In other experiments in which standard " conditioning " stimulations were followed by " test " stimulations of variable frequency, the latency of sweat emergence was observed to vary inversely as the frequency of " test " stimulation within a specified frequency range. At low frequencies, no sweat emergence was seen. These observations provide independent direct evidence that sweat is reabsorbed at least grossly in the inactive gland and indicate that the reabsorptive process is slow relative to the process of sweat formation, but that the rate of the latter can be varied widely. Thus a balance between formation and reabsorption can be established at low stimulation frequencies.

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† Because of the failure to observe a reciprocal relation between the rate of excretion of potassium and the rate of excretion of sodium in the sweat, any postulated cholinergic action on cation transfers in the sweat gland must differ from the action of acetylcholine on the transport of sodium and potassium across nerve, muscle, and erythrocyte membranes.

Recently evidence for the formation of a precursor secretion differing from the final external product has been found in a study of potassium, sodium, and urea isotope exchange across the ducts of the salivary gland (Burgen and Seeman, 1958). Also, there has been a revival of interest in the electrophysiology of the salivary glands, and intracellular microelectrodes have been used to record the membrane potentials of single salivary gland cells (Lundberg, 1958). A resting potential of a little more than 30 mv has been recorded over both the outer (interstitial) and inner (acinar) cell faces, the cell interior being negative to the exterior. With stimulation of the parasympathetic nerve supply to the gland or after injection of acetylcholine into the arterial bloodstream, the outer cell face hyperpolarizes, the potential difference across it increasing from 30 to 60 mv. The magnitude and direction of this secretory hyperpolarization proved to be independent of the experimentally manipulated level of the resting potential, but the response was almost entirely suppressed when chloride ions were replaced by nitrate ions in a perfused sublingual gland preparation. Therefore, an active transport of chloride from the interstitial fluid to the cell interior was regarded tentatively as a primary secretory event responsible for the generation of the secretory hyperpolarization of the outer cell membrane and, in turn, for the secondary movement of cations and water to meet the requirements for electroneutrality and osmotic equilibrium, respectively. Unfortunately, electrochemical data bearing on sweat gland physiology are too deficient to permit even a tentative comparable theory of sweat secretion.

D. Apocrine Sweat Glands

Apocrine sweat glands are derived from the epithelium of the hair follicles into which their secretions empty. They are larger than eccrine glands with more deeply placed subdermal coils and wider tortuous ducts. Phylogenetically, they are more primitive than eccrine glands ; and in animals other than man, in which the eccrine glands are limited to the hairless footpads, the apocrine glands are widely distributed over the general body surface and exhibit considerable variation in structure and in the character of their secretions. They can be activated by acetylcholine, but recent studies of apocrine sweat secretion in the horse indicate that the glands normally respond to humoral adrenalin and not to the activation of either cholinergic or adrenergic secretory nerves (Evans and Smith, 1956; Bell and Evans, 1956; Evans *et al.*, 1956). In man, in whom the eccrine glands have evolved as an elaborate, unique component of the body temperature-regulating system, the apocrine glands are rudimentary, occurring only in axillary, perimammary, midline abdominal, perineal, pubic, genital and perianal areas, and, in modified form, in the nasal vestibule and external ear. Although, classically, the apocrine glands are considered to produce a holocrine secretion alternating with a true merocrine secretion, Shelley and Hurley (1953) regard the only function of the apocrine glands to be the periodic production in response to adrenergic stimulation of small amounts of a semiholocrine milky secretion containing protein, lipid, reducing sugars, ammonia, undefined odorous substances, iron (see Section VI, C), and copper. Current interpretations of apocrine function are reviewed by Rothman (1954) and Kuno (1956). Kuno believes there is no fundamental difference between eccrine and apocrine glands, both producing mixed secretions, but with a high ratio of the merocrine to holocrine component in eccrine sweat and a lower ratio in apocrine sweat. With respect to the latter, apart from the demonstrated iron content, there is no body of quantitative chemical data on the mineral composition of the apocrine secretion, and therefore the contribution of the apocrine component to the total dermal output of minerals from the body remains to be evaluated.

E. Sebaceous Glands

There have been numerous studies of factors affecting the activity of the sebaceous glands and detailed analyses of the lipid, alcohol, and hydrocarbon constituents of their secretion, but there has been little, if any, interest in the mineral components of sebum. The glands are multilobulated solid cell masses which are derived from, are contiguous to, and empty into hair follicles. They produce a true necrobiotic holocrine secretion in that the gland cells disintegrate centrally and their entire contents are discharged into the duct and hair follicle and ultimately onto the horny layer of the skin. It is unlikely that the sebaceous glands have any significant role in the excretion of minerals from the body, although, as noted in an earlier section of this chapter, sebum facilitates the absorption of lead, tin, copper, arsenic, bismuth, antimony, mercury, and doubtless other metals which form lipid-soluble complexes with fatty acids.

F. Epidermal Desquamation, Hair, and Nails

Epidermal keratinization involves dehydration and complete cellular disintegration. The dead keratinized cells are continuously fragmented, shed from the skin surface, and in part removed as hair and nail clippings. The mineral losses accompanying this desquamative process cannot be measured precisely because of the difficulty of differentiating the true constituents of the shed keratinized flakes from the contaminating constituents of the various skin gland secretions and the diffusion component of the dermal excretory output. The high concentration of iron in the sediment of centrifuged sweat and the loss of various substances from the nonsweating skin have been discussed in the section on eccrine sweat. Although such losses have been referred to as desquamative, it would be more accurate to restrict this designation to data obtained by direct analysis of washed epidermal scales, hair, and nails. Hair is the best studied representative of these three keratinous tissues and has proved to be the site of accumulation, retention, and excretion of many physiological and foreign elements presented to the follicle internally and to the shaft externally (Rothman and Schaaf, 1929 ; Goldblum *et al.*, 1953 ; Flesch, 1954). Among these the following have been found in amounts exceeding 100 mg per kg of hair : calcium, magnesium, copper, iron, zinc, silicon, chlorine, sulfur, and phosphorus. Manganese, aluminum, lead, cobalt, nickel, boron, and chromium have been found in amounts ranging from 1 to 100 mg per kg of hair ; and arsenic, titanium, strontium, silver, bromine, iodine, and uranium have been demonstrated in trace amounts. Mercury, antimony, bismuth, thallium, fluorine, and selenium have been found in hair only after systemic administration.

The combination of heavy metals with the sulfhydryl groups of precursors of keratin and the subsequent incorporation of these metals into metabolically inactive keratin make it necessary to consider hair, nails, and epidermis as excretory channels. For example, hair is an important channel for the excretion of lead, selenium, and perhaps copper ; it is a major route for the excretion of arsenic, and as such it is important in the diagnosis of, if not in the intrinsic defense against, arsenic poisoning. The precise effectiveness of keratinous tissues as excretory pathways depends on genetic, nutritional, hormonal, and other physiological, as well as external, chemical, and physical influences on the rate of growth and shedding of hair (Flesch, 1954 ; Chase, 1954), nails (Lewis, 1954 ; Babcock, 1955) and cornified epidermis (Pinkus, 1954 ; Leblond and Walker, 1956).

External contamination may account for variations in analytical data with respect to such omnipresent elements as calcium, magnesium, silicon, and chloride ; but contamination is a most unlikely explanation for the large total content of these substances in hair, despite the fact that the chemical nature of their association with follicular protein and keratin is not clear. External exposure to copper leads to striking accumulations in hair and nails as a result of the combination of copper with cystine. The normally high copper content of hair is related to its specific function in pigment formation (Marston, 1952 ; Lorincz, 1954) and the keratinization process (Marston, 1946, 1952 ; Rothman, 1954). Iron, which is required for the normal growth of nails, and probably of hair as well, is particularly concentrated in red hair where it is a constituent of the red pigment (Dutcher and Rothman, 1951). The significance of the high zinc content of hair, nail, and epidermal keratin is not known ; nor is any reason known for the normal occurrence of lead in hair other than the fact that lead complexes strongly with hair keratin even on external contact. After systemic administration or feeding, 50 % or more of the body lead increment is accumulated in the hair (Kraut and Weber, 1944).

Carruthers and Suntzeff (1953) report the presence of 3.22 mg of potassium, 1.23 mg of sodium, 0.16 mg of calcium, 0.19 mg of magnesium, 0.024 mg of zinc and 0.0054 mg of copper per gram of wet separated epidermal tissue. In addition the skin contains aluminum, silicon, and iodine (Lobitz, 1954). Microincineration of the epidermis (Scott, 1933 ; MacCardle *et al.*, 1943) shows a striking accumulation of calcium, magnesium, and silicon in the superficial epidermal layers, the deposit moving toward the surface and increasing in amount with advancing age. The concentration of sulfur in the epidermis is more than twice as great as in the skin as a whole, and greater than in hair and nails. Normally sulfur is present almost entirely as cystine and cysteine, but abnormally, half of it may be present as methionine (Lobitz, 1954). Findings of other workers and reasons for variation in analytical data on skin and other tissues have been reviewed by Manery (1954, and Vol. I, Chapter 13).

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